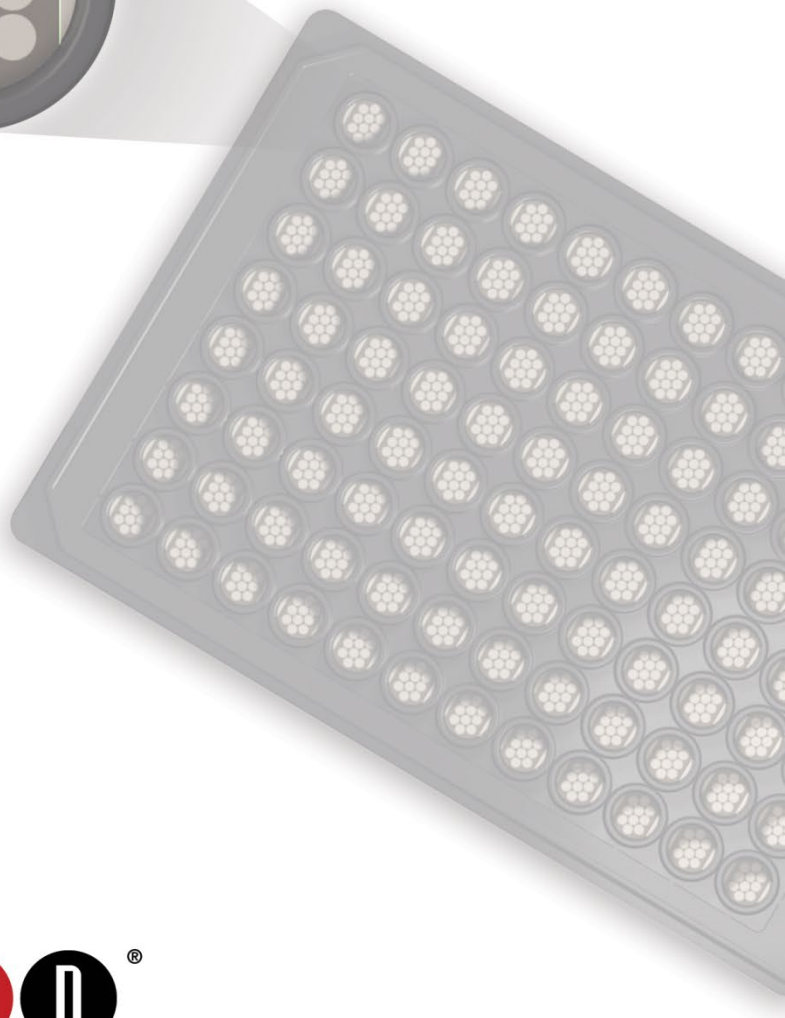
Cytokine Panel 2 (human) Kits

IL-17A/F, IL-17B, IL-17C, IL-17D, IL-1RA, IL-3, IL-9, TSLP

V-PLEX®



	V-PLEX®	V-PLEX Plus
Multiplex Kits	K15084D	K15084G
Individual Assay Kits		
Human IL-17A/F	K151WND	K151WNG
Human IL-17B	K151WOD	K151WOG
Human IL-17C	K151WPD	K151WPG
Human IL-17D	K151WQD	K151WQG
Human IL-1RA	K151WTD	K151WTG
Human IL-3	K151WZD	K151WZG
Human IL-9	K151XCD	K151XCG
Human TSLP	K151XED	K151XEG

MSD Cytokine Assays

Cytokine Panel 2 (human) Kits

IL-17A/F, IL-17B, IL-17C, IL-17D, IL-1RA, IL-3, IL-9, TSLP

For use with human cell culture supernatants, serum, plasma, and urine.

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®
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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[5] 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. Most Individual V-PLEX assays are provided on MSD's single-spot, 96-well plates. The remaining are provided on the multiplex panel plate.

The Cytokine Panel 2 (human) measures eight cytokines that are important in key inflammatory pathways. Improper regulation of their proinflammatory activities contributes to numerous pathogenic conditions such as rheumatoid arthritis, psoriasis, multiple sclerosis, Crohn's disease, asthma, and DIRA (deficiency of the interleukin-1 receptor antagonist). As a result of their association with autoimmune and autoinflammatory diseases, these biomarkers are the subjects of drug discovery projects, diagnostics development, and basic research. The biomarkers constituting the panel are: IL-17A/F, IL-17B, IL-17C, IL-17D, IL-1RA, IL-3, IL-9, and TSLP.

Principle of the Assay

MSD cytokine assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The assays in the Cytokine 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 IL-17A/F, IL-17B, IL-17C, IL-17D, IL-3, IL-9, and TSLP assays are provided on 10-spot MULTI-SPOT plates (Figure 1); the individual IL-1RA assay is provided on Small Spot plates (Figure 2). The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD GOLD™ 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.[5]

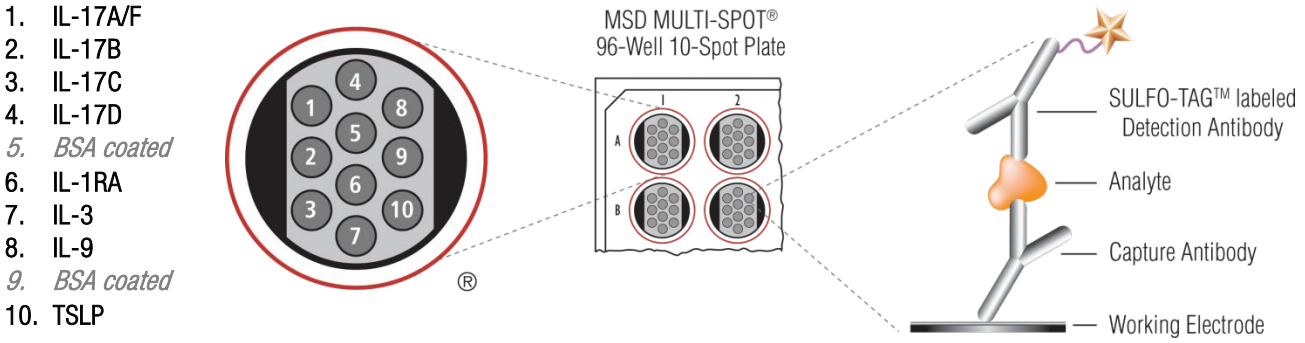

Figure 1. Multiplex plate spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

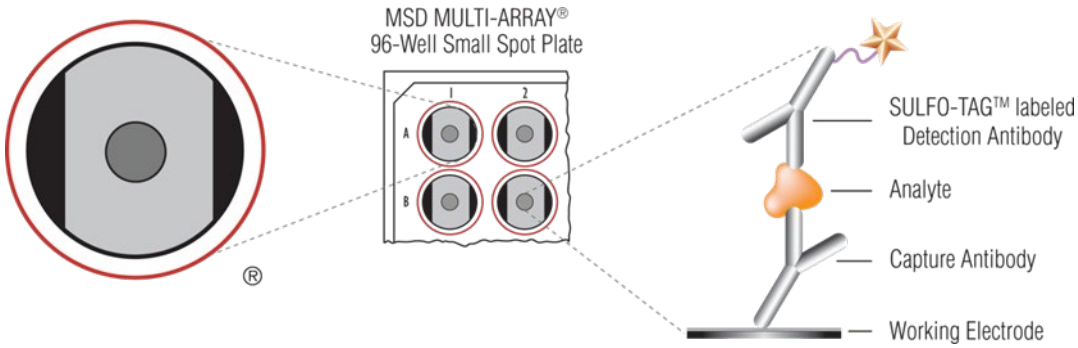

Figure 2. Small Spot plate diagram showing placement of analyte capture antibodies.

Kit Components

Cytokine Panel 2 (human) assays are available as a multiplex kit, as individual assay kits, or as custom V-PLEX kits with subsets of assays selected from the full panel. V-PLEX Plus kits include additional items (controls, wash buffer, and plate seals). See below for details.

Reagents Supplied With All Kits

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

Reagent	Storage	Catalog #	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	
Cytokine Panel 2 (human) Calibrator Blend	2–8 °C	C0084-2	1 vial	1 vial	5 vials	25 vials	Eight recombinant human proteins in diluent, buffered and lyophilized. Individual analyte concentration is provided in the lot-specific certificate of analysis (COA).
Diluent 43	≤-10 °C	R50AG-1	10 mL	1 bottle			Diluent for samples and calibrator; contains protein, blockers, and preservatives.
		R50AG-2	50 mL		1 bottle	5 bottles	
Diluent 3	≤-10 °C	R51BA-4	5 mL	1 bottle			Diluent for detection antibody; contains protein, blockers, and preservatives.
		R51BA-5	25 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.

V-PLEX Plus Kits: Additional Components

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

Reagents	Storage	Catalog #	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	
Cytokine Panel 2 (human) Control 1*	2–8 °C	C4084-1	1 vial	1 vial	5 vials	25 vials	Multi-analyte controls in a non-human matrix, buffered, lyophilized, and spiked with recombinant human analytes. The concentration of the controls is provided in the lot-specific COA.
Cytokine Panel 2 (human) Control 2*	2–8 °C	C4084-1	1 vial	1 vial	5 vials	25 vials	
Cytokine Panel 2 (human) Control 3*	2–8 °C	C4084-1	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 Cytokine Panel 2 (human) Control Pack (catalog # C4084-1)

Kit-Specific Components

Table 3. Components that are supplied with specific kits

Plates	Storage	Part #	Size	Quantity Supplied 1-Plate Kit	5-Plate Kit	25-Plate Kit	Description
Cytokine Panel 2 (human) SECTOR® Plate	2–8 °C	N05084A-1	10-spot	1	5	25	96-well plate, foil sealed, with desiccant.
Human IL-1RA SECTOR Plate	2–8 °C	L451WTA-1	Small Spot	1	5	25	

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

SULFO-TAG Detection Antibody	Storage	Catalog #	Size	Quantity Supplied 1-Plate Kit	5-Plate Kit	25-Plate Kit	Description
Anti-hu IL-17A/F Antibody (50X)	2–8 °C	D21WN-2	75 µL	1			SULFO-TAG™ conjugated antibody
		D21WN-3	375 µL		1	5	
Anti-hu IL-17B Antibody (50X)	2–8 °C	D21WO-2	75 µL	1			SULFO-TAG conjugated antibody
		D21WO-3	375 µL		1	5	
Anti-huIL-17C Antibody (50X)	2–8 °C	D21WP-2	75 µL	1			SULFO-TAG conjugated antibody
		D21WP-3	375 µL		1	5	
Anti-hu IL-17D Antibody (50X)	2–8 °C	D21WQ-2	75 µL	1			SULFO-TAG conjugated antibody
		D21WQ-3	375 µL		1	5	
Anti-hu IL-1RA Antibody (50X)	2–8 °C	D21WT-2	75 µL	1			SULFO-TAG conjugated antibody
		D21WT-3	375 µL		1	5	
Anti-hu IL-3 Antibody (50X)	2–8 °C	D21WZ-2	75 µL	1			SULFO-TAG conjugated antibody
		D21WZ-3	375 µL		1	5	
Anti-hu IL-9 Antibody (50X)	2–8 °C	D21XC-2	75 µL	1			SULFO-TAG conjugated antibody
		D21XC-3	375 µL		1	5	
Anti-hu TSLP Antibody (50X)	2–8 °C	D21XE-2	75 µL	1			SULFO-TAG conjugated antibody
		D21XE-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

- ❑ Cytokine Panel 2 (human) Control Pack, available for separate purchase from MSD, catalog # C4084-1 (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.

⚠ CAUTION: IL-1RA is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

Best Practices and Technical Hints

- Mixing or substituting reagents from different sources or different kit lots is not recommended. 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 diluents to room temperature in a 20–26 °C water bath prior to use. If a controlled water bath is not available, thaw at room temperature. Diluents may also be thawed overnight at 2-8 °C. 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 and mix by vortexing after each dilution.
- 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 during all pipetting steps as they may lead to variable results. Bubbles introduced when adding read buffer may interfere with signal detection.
- Plate shaking should be vigorous, with a rotary motion between 500–1,000 rpm. Binding reactions may reach equilibrium sooner if shaken in the middle of this range (~700 rpm) or above.
- Use reverse pipetting when necessary to avoid introduction of bubbles. For empty wells, pipette to the bottom corner. Do not touch the pipette tip to the bottom of the wells when pipetting into the MSD plate.
- Gently tap the plate on a paper towel to remove residual fluid after washing.
- 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.
- Read buffer should be at room temperature (20-26 ºC) before adding it to the plate.
- Do not shake the plate after adding read buffer.
- Keep time intervals consistent between addition of read buffer and reading the plate to improve inter-plate precision. It is recommended that a MSD instrument be prepared to read a plate before adding Read Buffer. Unless otherwise directed, read the plate as soon as possible after adding read buffer.
- If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay.

Reagent Preparation

Bring all reagents to room temperature.

Important: Upon first thaw, separate Diluent 43 and Diluent 3 into suitably-sized aliquots before refreezing. After thawing Diluent 43, you may see precipitate in the solution. Mix or vortex the diluent and proceed with the assay. Any remaining precipitate will not compromise assay performance.

⚠ CAUTION: IL-1RA is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

Prepare Calibrator Dilutions

MSD supplies a multi-analyte lyophilized calibrator that yields the recommended highest calibrator concentration when reconstituted in 1,000 µL of Diluent 43. (For individual assays that do not saturate at the highest calibrator concentration, the calibration curve can be extended by creating a more concentrated highest calibrator. In this case, follow the steps below using 250 µL instead of 1,000 µL of Diluent 43 when reconstituting the lyophilized calibrator.) Keep reconstituted calibrator and calibrator solutions on wet ice until use.

To prepare 7 calibrator solutions plus a zero calibrator for up to 4 replicates (Figure 3):

1) Prepare the highest calibrator (Calibrator 1) by adding 1,000 µL of Diluent 43 to the lyophilized calibrator vial. After reconstituting, invert at least 3 times (do not vortex). Let the reconstituted solution equilibrate at room temperature for 15-30 minutes and then vortex briefly using short pulses.
2) Prepare the next calibrator by transferring 100 µL of the highest calibrator to 300 µL of Diluent 43. Mix well by vortexing. Repeat 4-fold serial dilutions 5 additional times to generate 7 calibrators.
3) Use Diluent 43 as the zero calibrator.

Note: Reconstituted calibrator is not stable when stored at 2–8 °C; however, it may be stored frozen at ≤-70 °C and is stable through three freeze–thaw cycles. 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.

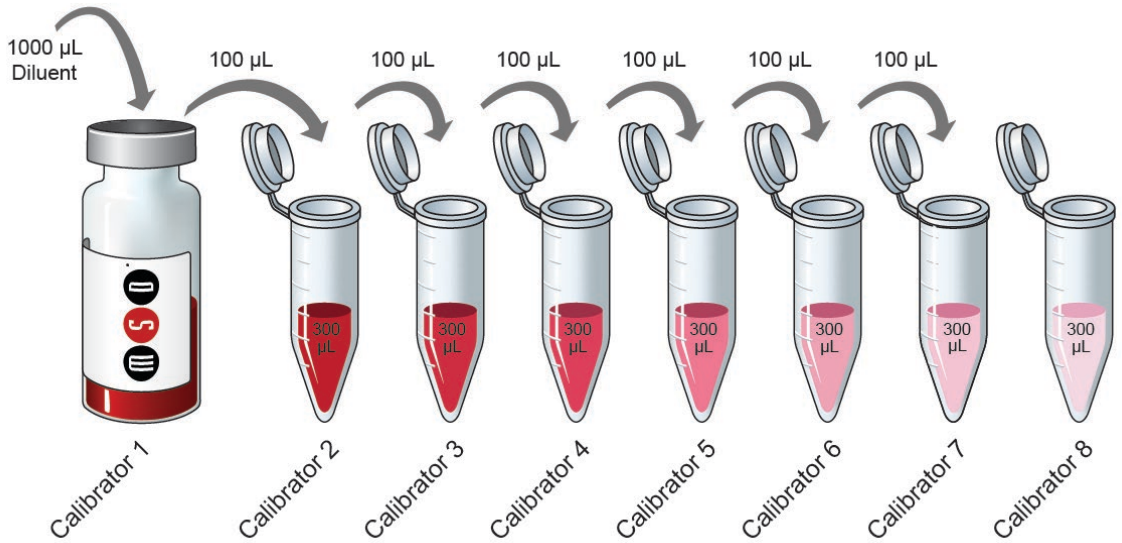

Figure 3. Dilution schema for preparation of Calibrator Standards

Sample Collection and Handling

Below are general guidelines for human sample collection, storage, and handling. If possible, use published guidelines.[1-4] 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 2,000g 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 ≤-10 ºC until needed. Repeated freezing and thawing of samples is not recommended. After thawing, centrifuge samples at 2,000g for 3 minutes to remove particulates prior to sample preparation.

Dilute Samples

Dilute samples with Diluent 43. For human serum, plasma, and urine samples, MSD recommends a minimum 4-fold dilution. For example, when running samples in duplicate, add 30 µL of sample to 90 µL of Diluent 43. We recommend running at least two replicates per sample. When running unreplicated samples, add 25 µL of sample to 75 µL of Diluent 43. You may conserve sample volume by using a higher dilution. Tissue culture supernatants may require additional dilution based on stimulation and analyte concentrations in the sample. The kit includes diluent sufficient enough for running samples in duplicates. Additional diluent can be purchased at www.mesoscale.com.

Prepare Controls

Three levels of multi-analyte lyophilized controls are available for separate purchase from MSD in the Cytokine Panel 2 (human) Control Pack, catalog # C4084-1. (Controls are included only in V-PLEX Plus kits.)

Reconstitute the lyophilized controls in 250 µL of Diluent 43. Do not invert or vortex the vials. Wait for a minimum of 15-30 minutes at room temperature before diluting controls 4-fold in Diluent 43. Vortex briefly using short pulses. Reconstituted controls may be stored frozen at ≤-70 °C and are stable through three freeze–thaw cycles. For the lot-specific concentration of each analyte in the control pack, refer to the supplied COA. You can also find a copy of the COA 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.

Multiplex Cytokine Panel 2 (human) kit

For one plate, combine the following detection antibodies and add to 2,520 µL of Diluent 3:

- ❑ 60 µL of SULFO-TAG Anti-hu IL-17A/F Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu IL-17B Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu IL-17C Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu IL-17D Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu IL-1RA Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu IL-3 Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu IL-9 Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu TSLP Antibody

Custom multiplex kits

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

Individual assay kits

For one plate, add 60 µL of the supplied detection antibody to 2,940 µL of Diluent 3.

Prepare Wash Buffer

MSD provides 100 mL of Wash Buffer as a 20X stock solution in the 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

Prepare Read Buffer T

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For one plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 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 (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Pre-wash plates before use as recommended on the assay protocol.

Protocol

Note: Follow **Reagent Preparation** before beginning this assay protocol.

STEP 1: Wash and Add Sample

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 50 µL of prepared samples, calibrators or controls per well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 2 hours.

Note: Washing the plate prior to sample addition is an optional step that may provide greater uniformity of results for certain assays. Analytical parameters, including limits of quantification, recovery of controls, and sample quantification, are not affected by washing the plate prior to sample addition.

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 of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 2 hours.

STEP 3: Wash and Read

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 150 µL of 2X Read Buffer T to each well. Analyze the plate on an MSD instrument. Incubation in Read Buffer T is not required before reading the plate.

Alternate Protocols

The suggestions below may be useful as alternate protocols; however, not all were tested using multiple kit lots.

- **Alternate Protocol 1, Extended Sample Incubation:** Incubating samples overnight at 2-8 ºC may improve sensitivity for some assays. See **Appendix A** for specific assays that may benefit from this alternate protocol.
- **Alternate Protocol 2, Reduced Wash:** For tissue culture samples, you may simplify the protocol by eliminating one of the wash steps. After incubating diluted sample, calibrator, or control, add detection antibody solution to the plate without decanting or washing the plate. See **Appendix A** for assay performance using this protocol.
- **Alternate Protocol 3, Dilute-in-Plate:** To limit sample handling, you may dilute samples and controls in the plate. For 4-fold dilution, add 37.5 µL of assay diluent to each sample/control well, and then add 12.5 µL of neat control or sample. Calibrators should not be diluted in the plate; add 50 µL of each calibrator directly into empty wells. Tests conducted according to this alternate protocol produced results that were similar to the recommended protocol (data not shown).
- **Alternate Protocol 4, Acid Treatment of Samples:** Spike recovery for some analytes in serum may have an improvement by using an acid treatment protocol to dissociate soluble binding receptors that may bind spiked in calibrator. The protocol and the spike recovery results from acid-treated samples are in **Appendix D**.

Validation

MSD's V-PLEX products are validated following fit-for-purpose principles[5] 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. During validation, each individual assay is analytically validated as a singleplex and is also independently evaluated as a multiplex component by running the full multiplex plate using only the single detection antibody for that assay. These results are compared with the results from the multiplex panel when using all detection antibodies. This demonstrates that each assay is specific and independent, allowing them to be multiplexed in any combination. The COA provided with each kit outlines the kit release specifications for sensitivity, specificity, accuracy, and precision.

- **Development**

  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 this 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 25%. Rigorous management of inter-lot reagent consistency and calibrator production results in typical inter-lot CVs below 10%. Validation lots are compared using controls and at least 30 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 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 are provided in this product insert). In addition to the matrices listed above, diseased patients sample, PBMCs, and/or cell lines that have been stimulated to generate elevated levels of analytes are tested. Results confirm measurement of native proteins at concentrations that are often higher than those found in individual native samples.

- **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 1) with the multi-analyte calibrator and assay-specific detection antibodies, and 2) 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.

  In addition to measuring the specificity of antibodies to analytes in the multiplex kit, specificity and interference from other related markers are tested during development. This includes evaluation of selected related proteins and receptors or binding partners.

- **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 Cytokine Panel 2 (human) were collected over five months of testing by 5 operators (49 runs in total). Calibration curve accuracy and precision were assessed for two kit lots. Representative data from one lot are presented below. (Data from individual assays are presented in **Appendix B**.) The multiplex panel was tested with individual detection antibodies to demonstrate that the assays are independent of one another. **Appendix C** compares results for each assay in the kit when the panel is run using the individual detection antibody versus all detection antibodies. The calibration curves were comparable. Calibration curves for each lot are presented in the lot-specific COA.

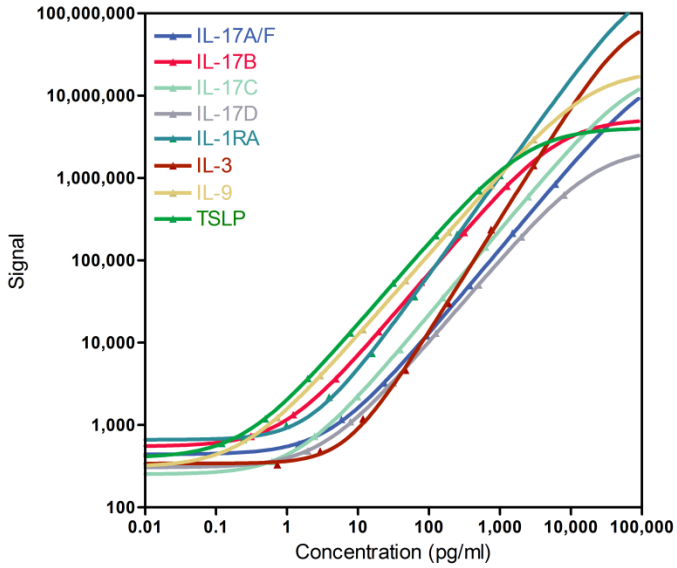

Figure 4. Typical calibration curves for the Cytokine Panel 2 (human) assay.

Sensitivity

The LLOD is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero calibrator). The LLOD shown below was calculated based on 60 runs.

The ULOQ is the highest concentration at which the CV of the calculated concentration is <20% and the recovery of each analyte is within 80% to 120% of the known value.

The LLOQ is the lowest concentration at which the CV of the calculated concentration is <20% and the recovery of each analyte is within 80% to 120% of the known value (75% to 125% for IL-17B, IL-1RA, IL-3, TSLP, IL-17D). 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 Cytokine Panel 2 (human) Kit

	Median LLOD (pg/mL)	LLOD Range (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
IL-17A/F	0.930	0.324-1.83	7.57	3,900
IL-17B	0.185	0.094-0.343	1.12	1,040
IL-17C	0.682	0.273-1.61	3.84	1,620
IL-17D	3.87	0.497-6.82	11.2	5,200
IL-1RA	1.12	0.142-6.47	9.19	650
IL-3	2.37	0.739-5.53	12.6	1,950
IL-9	0.311	0.055-1.33	2.23	975
TSLP	0.063	0.013-0.179	0.460	325

Precision

Controls were made by spiking calibrator into non-human matrix at three levels within the quantitative range of the assay. Analyte levels were measured by 5 operators using a minimum of 3 replicates on 55 runs. Results are shown below. While a 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 10%.

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 15 runs within a single kit lot

Inter-lot %CV is the variability of controls across 3 kit lots (total of 60 runs)

Table 6. Intra-run and Inter-run %CVs for each analyte in the Cytokine Panel 2 (human) Kit

	Control	Average Conc. (pg/mL)	Average Intra-run %CV	Inter-run %CV	Inter-lot %CV
IL-17A/F	Control 1	6,019	1.5	4.8	0.9
	Control 2	766	1.9	4.6	0.9
	Control 3	67	3.9	8.0	1.1
IL-17B	Control 1	546	2.7	10.6	0.9
	Control 2	82	2.4	14.8	5.3
	Control 3	141	3.2	12.2	0.1
IL-17C	Control 1	2,205	5.2	8.1	2.3
	Control 2	267	6.3	7.7	2.4
	Control 3	67	5.3	10.8	0.7
IL-17D	Control 1	5,588	4.5	11.1	1.3
	Control 2	540	4.7	12.3	5.0
	Control 3	137	5.8	13.1	3.5
IL-1RA	Control 1	664	4.3	8.0	1.7
	Control 2	136	4.4	9.4	4.5
	Control 3	46	5.0	10.2	4.4
IL-3	Control 1	3,029	3.8	7.2	5.8
	Control 2	654	4.1	9.6	4.9
	Control 3	214	5.3	11.6	8.1
IL-9	Control 1	1,352	3.0	5.9	0.9
	Control 2	353	2.9	6.4	0.7
	Control 3	66	2.7	7.3	1.6
TSLP	Control 1	592	4.5	10.6	4.4
	Control 2	88	5.6	9.5	4.2
	Control 3	11	6.4	7.5	4.9

Dilution Linearity

To assess linearity, normal human serum, EDTA plasma, heparin plasma, citrate plasma, and urine from a commercial source as well as cell culture supernatants were spiked with recombinant calibrators and diluted 8-fold, 16-fold, and 32-fold before testing. Percent recovery at each dilution level was normalized to the dilution-adjusted, 4-fold concentration. The average percent recovery is based on samples within the quantitative range of the assay.

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

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

		IL-17A/F		IL-17B		IL-17C		IL-17D	
Sample Type	Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Serum (N=10)	8	98	91-104	110	103-133	134	106-207	101	98-105
	16	98	92-102	119	110-146	157	128-208	97	89-105
	32	96	89-103	129	114-139	198	150-319	96	91-103
EDTA Plasma (N=10)	8	91	80-98	108	94-121	112	90-155	98	85-126
	16	88	77-97	112	90-137	119	94-173	100	86-120
	32	86	76-97	124	96-160	130	101-169	111	81-141
Citrate Plasma (N=10)	8	100	95-112	195	164-360	132	93-167	100	88-138
	16	101	87-119	464	330-807	182	145-274	109	87-182
	32	100	88-131	963	671-1,351	300	224-437	130	96-222
Urine (N=10)	8	105	73-152	142	98-209	131	115-172	101	89-116
	16	98	69-121	163	111-229	153	124-197	93	84-103
	32	94	67-121	203	135-253	192	149-291	89	71-99
Heparin Plasma (N=10)	8	100	91-114	103	90-121	104	88-142	109	94-142
	16	102	89-123	107	87-134	100	87-156	121	96-146
	32	102	94-108	103	91-128	94	80-105	131	115-147
Cell Culture Supernatant (N=6)	8	98	91-119	107	103-112	120	101-169	105	101-122
	16	85	48-110	109	104-115	98	64-123	100	86-115
	32	89	77-107	117	110-122	112	65-123	107	98-122

Table 7. continued.

		IL-1RA		IL-3		IL-9		TSLP	
Sample Type	Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Serum (N=10)	8	100	94-110	115	109-133	111	91-173	131	125-158
	16	100	92-112	128	120-148	136	120-201	153	140-179
	32	103	97-114	149	137-163	162	120-184	174	161-204
EDTA Plasma (N=10)	8	90	75-103	92	86-98	100	81-120	117	111-132
	16	82	70-95	87	77-99	107	82-151	136	121-157
	32	83	69-94	94	76-103	115	86-150	159	136-201
Citrate Plasma (N=10)	8	98	93-108	119	109-144	99	86-121	127	119-188
	16	97	88-115	143	121-165	127	112-165	140	122-195
	32	93	73-105	161	144-186	173	143-205	138	107-149
Urine (N=10)	8	109	87-166	105	96-130	106	84-126	118	105-133
	16	100	80-144	108	100-128	128	110-164	130	102-160
	32	112	82-162	132	121-149	166	121-201	158	136-183
Heparin Plasma (N=10)	8	111	89-147	95	91-99	101	89-109	93	87-97
	16	118	90-178	89	84-93	103	93-112	90	86-95
	32	127	88-192	91	83-94	105	95-113	91	88-94
Cell Culture Supernatant (N=6)	8	94	90-99	93	90-97	98	95-103	96	90-102
	16	90	86-94	87	83-93	99	88-104	92	90-94
	32	97	93-105	91	89-93	102	96-108	97	92-100

Note: Citrate plasma exhibited variable recovery for some assays (data shown above). Further optimization may be required for this sample type.

Spike Recovery

Spike recovery measurements were evaluated for different sample types throughout the quantitative range of the assays. Multiple individual human samples (serum, EDTA plasma, heparin plasma, citrate plasma, and urine) were obtained from a commercial source. These samples, along with cell culture supernatants, were spiked with calibrators at three levels (high, mid, and low) then diluted 4-fold. The average % recovery for each sample type is reported along with the % recovery range.

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

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

	Serum (N=81; 27 Samples)		Urine (N=30; 10 samples)		Cell Culture Supernatants (N=18; 6 samples)	
	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
IL-17A/F	107	64 - 176	97	84 - 114	97	95 - 102
IL-17B	74	32 - 107	130	61 - 181	74	70 - 76
IL-17C	41	23 - 61	93	57 - 104	92	79 - 101
IL-17D	113	81 - 128	88	48 - 115	100	98 - 103
IL-1RA	98	66 - 135	88	52 - 134	102	99 - 106
IL-3	59	42 - 87	97	84 - 111	93	87 - 99
IL-9	65	33 - 109	100	87 - 107	90	88 - 93
TSLP	87	30 - 106	124	79 - 176	92	89 - 96

	Citrate Plasma (N=18; 6 samples)		Heparin Plasma (N=15; 5 samples)		EDTA Plasma (N=74; 26 samples)	
	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
IL-17A/F	102	95 - 107	88	75 - 95	104	83 - 121
IL-17B	62	52 - 70	6	5-8	82	63 - 101
IL-17C	28	23 - 37	19	10-30	68	35 - 82
IL-17D	112	94 - 127	55	4-74	94	77 - 155
IL-1RA	91	77 - 126	101	73 - 128	88	67 - 107
IL-3	49	33 - 61	43	33 - 58	69	51 - 77
IL-9	43	37 - 49	35	25 - 51	92	43 - 110
TSLP	63	58 - 68	79	48 - 99	71	57 - 90

Note: Improvement in spike recovery for some analytes (specifically IL-3, IL-9, and IL-17C) in serum can be achieved utilizing an acid treatment protocol to dissociate soluble binding receptors that may bind spiked in calibrator. See Appendix D for the acid treatment protocol and the results.

Specificity

To assess specificity, each assay antibody set in the panel was tested individually. Nonspecific binding was also evaluated with additional recombinant human analytes (bFGF, CRP, Eotaxin, Eotaxin-3, Flt-1, G-CSF, GM-CSF, ICAM-1, IFN-γ, IL-10, IL-12/23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A/F, IL-17A, IL-17F, IL-17B, IL-17C, IL-17D, IL-1RA, IL-1α, IL-1β,IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, PIGF, SAA, TARC, Tie-2, TNF-α, TNF-β, TSLP, VCAM-1, VEGF, VEGF-C, VEGF-D). Nonspecific binding was less than 0.5% for all assays in the kit.

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

Stability

The reconstituted calibrator, reconstituted controls, and diluents were tested for freeze–thaw stability. Results (not shown) demonstrated that reconstituted calibrator, reconstituted controls, and diluents can go through three freeze–thaw cycles without significantly affecting the performance of the assay. Reconstituted calibrator and controls must be stored frozen at ≤-70ºC. Partially used MSD plates may be sealed and stored up to 30 days at 2–8ºC in the original foil pouch with desiccant. Results from control measurements changed by ≤30% after partially used plates were stored for 30 days. The validation study includes a real-time stability study with scheduled performance evaluations of complete kits for up to 54 months from date of manufacture.

Calibration

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

MSD reference calibrators for the following analytes were evaluated against the NIBSC/WHO International Standards. The ratios of International Units of biological activity per mL (IU/mL) of NIBSC standard relative to pg/mL of MSD calibrator are shown in the table below. To convert MSD concentrations to biological activity relative to the WHO International Standard, multiply the MSD concentration by the ratio provided.

Table 9. Ratios of International Units (IU/mL) relative to MSD Calibrators (pg/mL)

	NIBSC/WHO Catalog Number	NIBSC (IU/mL): MSD (pg/mL)
IL-1RA	92/644	0.0070
IL-3	91/510	0.0048
IL-9	91/678	0.0183

Tested Samples

Normal Samples

Commercially available normal human serum, EDTA plasma, heparin plasma, citrate plasma, and urine samples were diluted 4-fold and tested. Results for each sample set are displayed below. Concentrations are corrected for sample dilution. Median and range are calculated from samples with concentrations at or above the LLOD. Percent detected is the percentage of samples with concentrations at or above the LLOD.

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

Sample Type	Statistic	IL-17A/F	IL-17B	IL-17C	IL-17D	IL-1RA	IL-3	IL-9	TSLP
Serum (N=25)	Median (pg/mL)	1.28	1.13	ND	9.30	191	ND	0.446	0.425
	Range (pg/mL)	0.7-1.9	0.2-2.6	NA	1.7-36.2	83.8-956	NA	0.1-0.9	0.3-2.0
	% Detected	8	92	0	92	100	0	100	96
EDTA Plasma (N=25)	Median (pg/mL)	20.1	1.06	1.48	74.5	185	24.6	3.59	0.748
	Range (pg/mL)	0.8-177	0.2-8.5	1.1-2.0	7.3-1,145	88.8-479	2.8-319	0.2-20.2	0.3-21.3
	% Detected	52	96	12	76	100	100	68	100
Heparin Plasma (N=25)	Median (pg/mL)	0.907	0.905	ND	4.48	139	3.64	0.298	0.748
	Range (pg/mL)	0.8-20.4	0.4-2.3	NA	1.6-50.1	78.6-1,176	2.7-33.9	0.1-0.6	0.3-4.9
	% Detected	20	56	0	72	100	12	56	96
Citrate Plasma (N=25)	Median (pg/mL)	14.5	2.23	3.45	68.2	168	121	1.40	5.47
	Range (pg/mL)	2.9-40.7	1.4-6.7	2.2-6.1	35-476	69.4-393	3.8-295	0.5-8.5	1.6-25.1
	% Detected	100	100	12	100	100	92	100	100
Urine (N=16)	Median (pg/mL)	4.27	4.40	13.0	6.90	1474	33.8	1.20	0.627
	Range (pg/mL)	0.7-25.3	0.3-8.7	1.3-27.9	2.1-19.7	65.3-14,064	13.8-57.8	0.4-2.9	0.2-4.3
	% Detected	50	31	31	38	63	31	56	44

ND = Non-detectable
NA = Range not available
% Detected = % of samples with concentrations at or above the LLOD

Stimulated Samples

A human lung cancer cell line (Calu-3) and a human T lymphoblastoid cell line (Jurkat E6.1) was stimulated for different time period with different stimulants as shown below. Supernatants were then isolated and tested. The dilution-adjusted concentrations (pg/mL) for each stimulation model are displayed below.

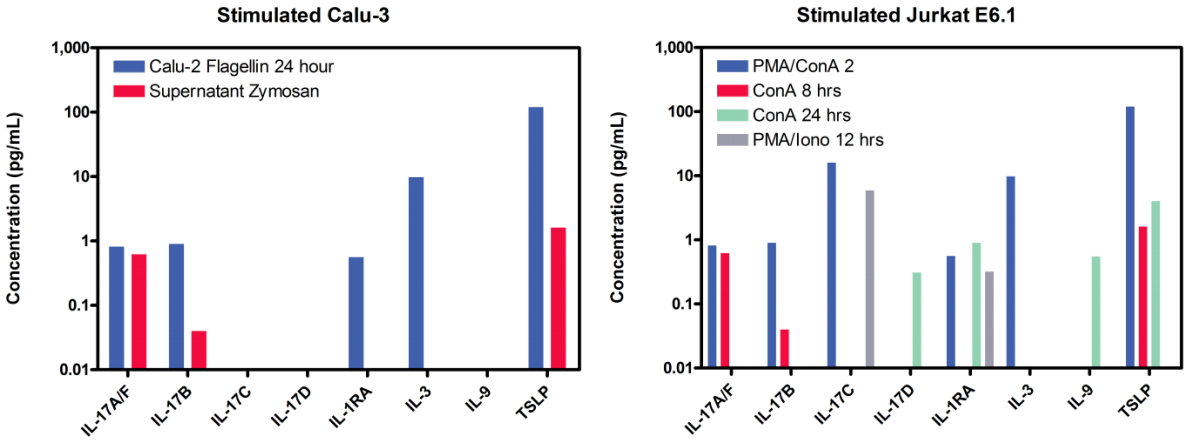

Figure 5. Effect of cell stimulation on cytokine production as measured in the Cytokine Panel 2 (human) Kit.

Samples from individuals with different disease conditions were tested for the Cytokine Panel 2 analyte levels. The dilution-adjusted concentrations (pg/mL) for each sample are displayed below.

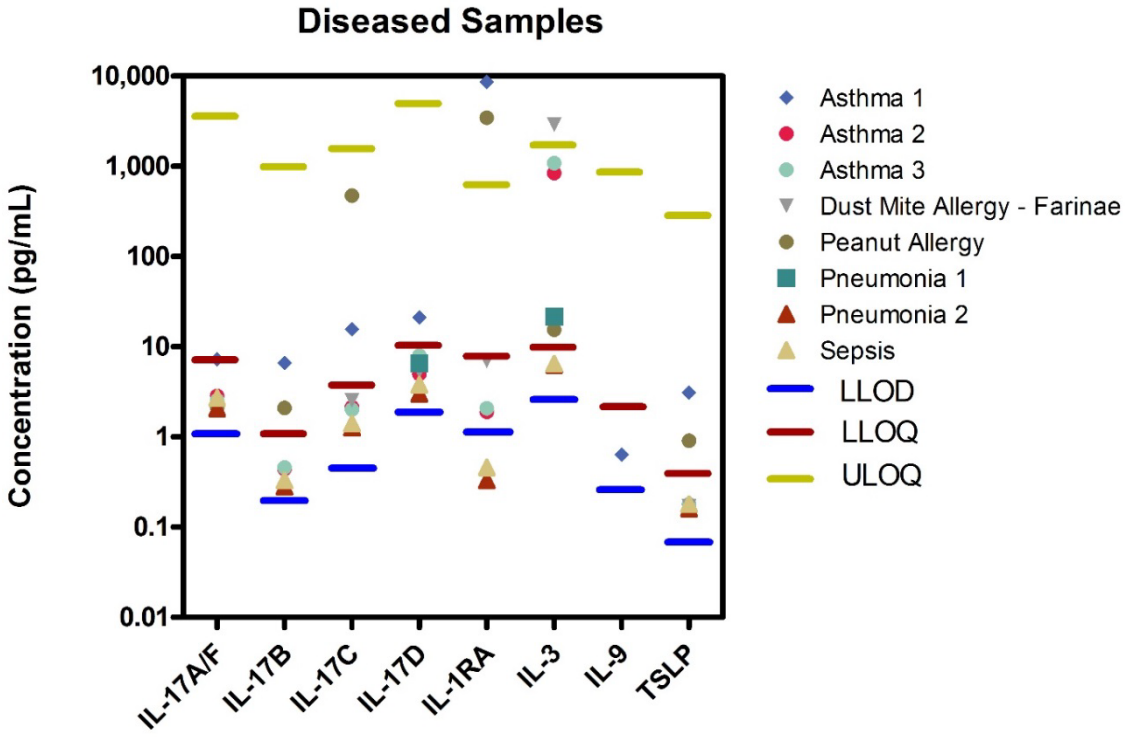

Figure 6. Expression level of each cytokine, measured using the Cytokine Panel 2 (human) Kit, in various diseased samples.

Assay Components

Calibrators

The assay calibrator blend uses the following recombinant human proteins:

Table 11. Recombinant human proteins used in the Calibrators

Calibrator	Expression System
IL-17A/F	Mouse cell line
IL-17B	Mouse cell line
IL-17C	*E.coli*
IL-17D	*E.coli*
IL-1RA	*E.coli*
IL-3	*E.coli*
IL-9	Insect cell line
TSLP	*E.coli*

Antibodies

Table 12. Antibody source species

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
IL-17A/F	Mouse Recombinant	Mouse Monoclonal	A
IL-17B	Mouse Monoclonal	Mouse Monoclonal	A
IL-17C	Mouse Monoclonal	Goat Polyclonal	A
IL-17D	Mouse Monoclonal	Mouse Monoclonal	A
IL-1RA	Mouse Monoclonal	Goat Polyclonal	A
IL-3	Mouse Monoclonal	Goat Polyclonal	A
IL-9	Mouse Monoclonal	Mouse Monoclonal	A
TSLP	Mouse Monoclonal	Mouse Monoclonal	A

References

1. Zhou H, et al. Collection, storage, preservation, and normalization of human urinary exosomes for biomarker discovery. Kidney. 2006;69:1471-6.
2. Thomas CE, et al. Urine collection and processing for protein biomarker discovery and quantification. Cancer Epidemiol Biomarkers & Prevention. 2010;19:953-9.
3. Schoonenboom NS, et al. Effects of processing and storage conditions on amyloid beta (1-42) and tau concentrations in cerebrospinal fluid: implications for use in clinical practice. Clin Chem. 2005;51:189-95.
4. Girgrah N, et al. Purification and characterization of the P-80 glycoprotein from human brain. Biochem J. 1988;256:351-6.
5. Lee JW, et al. Fit-for-purpose method development and validation for successful biomarker measurement. Pharm Res. 2006;23:312-28.

Appendix A

Calibration curves below illustrate the relative sensitivity for each assay using protocols defined in **Alternate Protocols** (see page 14): Reference Protocol (2-hour sample incubation/2 wash steps, blue curve), Alternate Protocol 1 (tissue culture: single wash, red curve), and Alternate Protocol 2 (overnight sample incubation, grey curve).

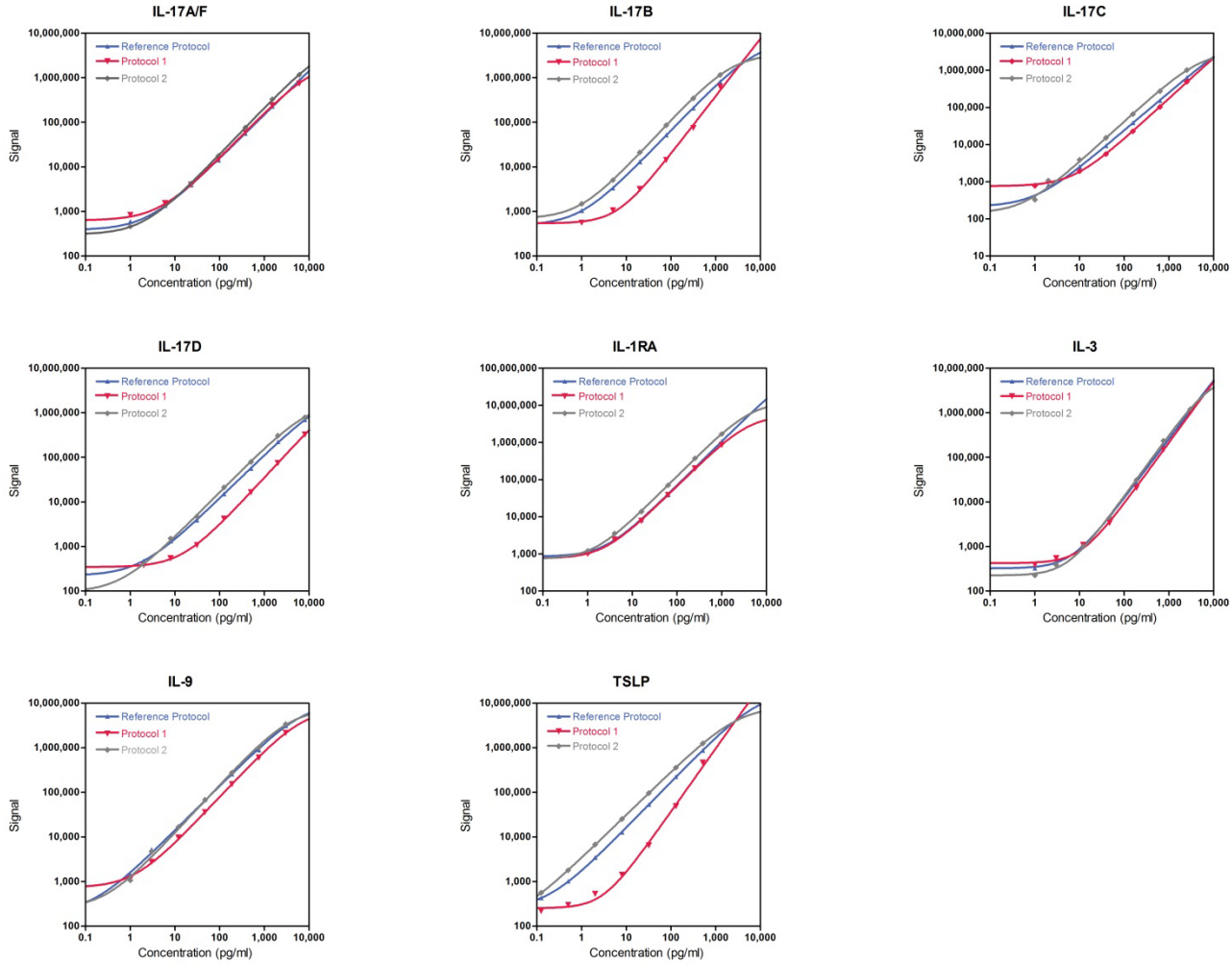

Table 13. Relative sensitivity when using alternate protocols

	LLOD Comparison (pg/mL)		
Assay	Reference Protocol	Protocol 1	Protocol 2
IL-17A/F	0.587	0.571	0.582
IL-17B	0.197	1.13	0.053
IL-17C	0.566	3.54	0.227
IL-17D	1.61	6.20	1.15
IL-1RA	0.487	0.226	0.316
IL-3	1.24	2.81	1.59
IL-9	0.165	1.51	0.075
TSLP	0.094	1.41	0.022

Appendix B

The calibration curves below compare assay performance when the assay is run as an individual assay on a single spot plate (red curve) vs. on the multiplex plate (blue curve).

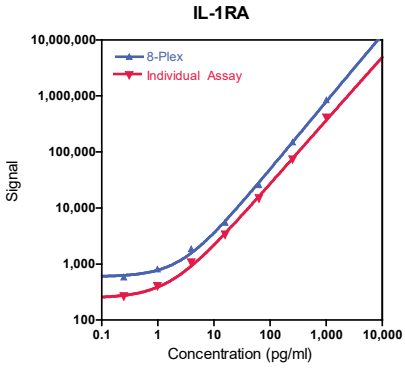

Table 14. Assay performance for individual and 8-plex assays

	LLOD (pg/mL)	
Assay	Individual	8-plex
IL-1RA	0.697	0.487

In general, assays in the single-spot format yielded a lower overall signal compared to the 8-plex format. The spots on single-spot plates have a larger binding surface than those on multiplex plates, but the same amount of calibrator was used for each test; therefore, the bound calibrator was spread over a larger surface area reducing the average signal.

Note: Assay performance for IL-17A/F, IL-17B, IL-17C, IL-17D, IL-3, IL-9, and TSLP is not included since the individual assay is run on multiplex plates.

Appendix C

The calibration curves below compare results for each assay in the panel when the assays were run on the 10-spot plate using all detection antibodies (blue curve) vs. running each assay using a single, assay-specific detection antibody (red curve).

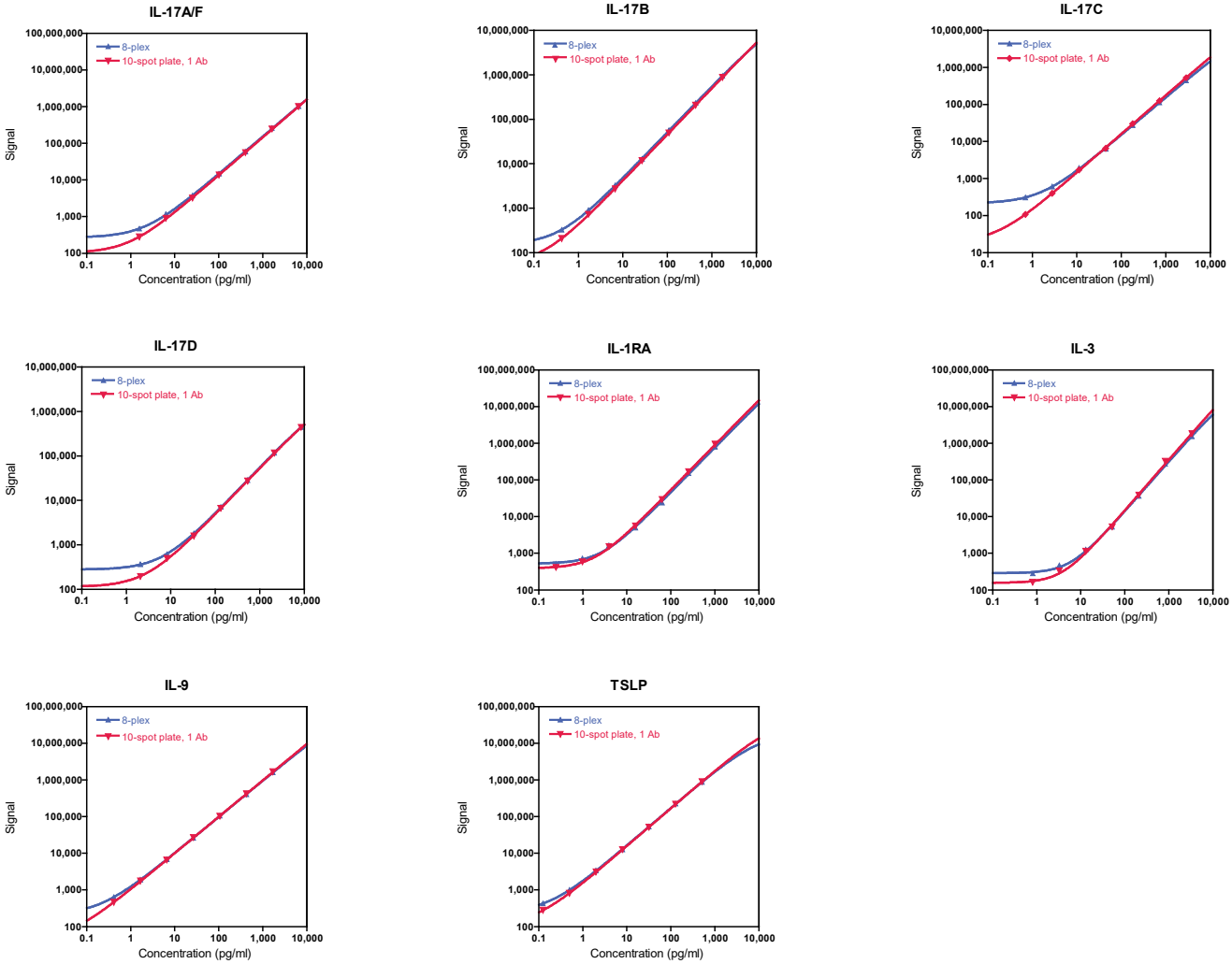

Table 15. LLODs for detection of a single antibody vs. blended antibodies

	LLOD (pg/mL)	
Assay	10-spot plate, 1 Ab	8-plex
IL-17A/F	0.634	0.561
IL-17B	0.208	0.176
IL-17C	0.621	0.711
IL-17D	1.86	1.46
IL-1RA	0.680	0.415
IL-3	1.92	2.60
IL-9	0.080	0.071
TSLP	0.053	0.052

Appendix D

Improvement in spike recovery for some analytes in serum can be achieved utilizing an acid treatment protocol to dissociate soluble binding receptors that may bind spiked in calibrator. Acid treatment of samples was done prior to spiking in recombinant calibrator. Results are shown in the table below.

Table 16. Effect of acid treatment of samples on spike recovery

	Serum (N=36; 12 samples)	
	Average %Recovery	% Recovery Range
IL-17A/F	107	99-119
IL-17B	133	113-143
IL-17C	89	84-95
IL-17D	134	53-155
IL-1RA	85	73-120
IL-3	90	82-95
IL-9	110	104-118
TSLP	96	79-101

Protocol for Acid Treatment of Samples

Materials Required (not supplied):

- ❑ Acid solution (1N HCl)
- ❑ Neutralization solution (1.2N NaOH/0.5M HEPES)

Protocol:

Each well will require 50 µL of diluted sample that has been activated by acid-treatment and subsequent neutralization.

1) To 40 µL serum, add 20 µL of 1N HCl, mix well and incubate for 10 minutes at room temperature.
2) Neutralize the acidified sample by adding 20 µL of 1.2N NaOH/0.5M HEPES; mix well.
3) Add 80 µL of Diluent 43 to neutralized sample. Add 50 µL of the neutralized sample to the plate.

If acid treatment results in viscous samples, step 2 can be performed on ice to minimize sample viscosity during acid treatment.

Summary Protocol

Cytokine Panel 2 (human) Kits

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Cytokine Panel 2 (human) assays.

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature.
- ❑ Prepare calibration solutions in Diluent 43 using the supplied calibrator:
    - Reconstitute the lyophilized calibrator blend.
    - Invert 3 times, equilibrate 15-30 minutes at room temperature.
    - Vortex briefly using short pulses.
    - Perform a series of 4-fold dilution steps and prepare a zero calibrator.
- ❑ Dilute the samples and controls 4-fold in Diluent 43 before adding to the plate.
- ❑ Prepare combined detection antibody solution by diluting each 50X detection antibody 50-fold in Diluent 3.
- ❑ Prepare 2X Read Buffer T by diluting 4X Read Buffer T 2-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 50 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 2 hours.

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 2X Read Buffer T.
- ❑ Analyze the plate on the MSD instrument.

***Note:** Washing the plate prior to sample addition is an optional step that may provide greater uniformity of results for certain assays. Analytical parameters including limits of quantification, recovery of controls, and sample quantification, are not affected by washing the plate prior to sample addition.

Catalog Numbers

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						
Cytokine Panel 2 (human)	K15084D-1	K15084D-2	K15084D-4	K15084G-1	K15084G-2	K15084G-4
Individual Assay Kits						
Human IL-17A/F	K151WND-1	K151WND-2	K151WND-4	K151WNG-1	K151WNG-2	K151WNG-4
Human IL-17B	K151WOD-1	K151WOD-2	K151WOD-4	K151WOG-1	K151WOG-2	K151WOG-4
Human IL-17C	K151WPD-1	K151WPD-2	K151WPD-4	K151WPG-1	K151WPG-2	K151WPG-4
Human IL-17D	K151WQD-1	K151WQD-2	K151WQD-4	K151WQG-1	K151WQG-2	K151WQG-4
Human IL-1RA	K151WTD-1	K151WTD-2	K151WTD-4	K151WTG-1	K151WTG-2	K151WTG-4
Human IL-3	K151WZD-1	K151WZD-2	K151WZD-4	K151WZG-1	K151WZG-2	K151WZG-4
Human IL-9	K151XCD-1	K151XCD-2	K151XCD-4	K151XCG-1	K151XCG-2	K151XCG-4
Human TSLP	K151XED-1	K151XED-2	K151XED-4	K151XEG-1	K151XEG-2	K151XEG-4

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

Plate Diagram