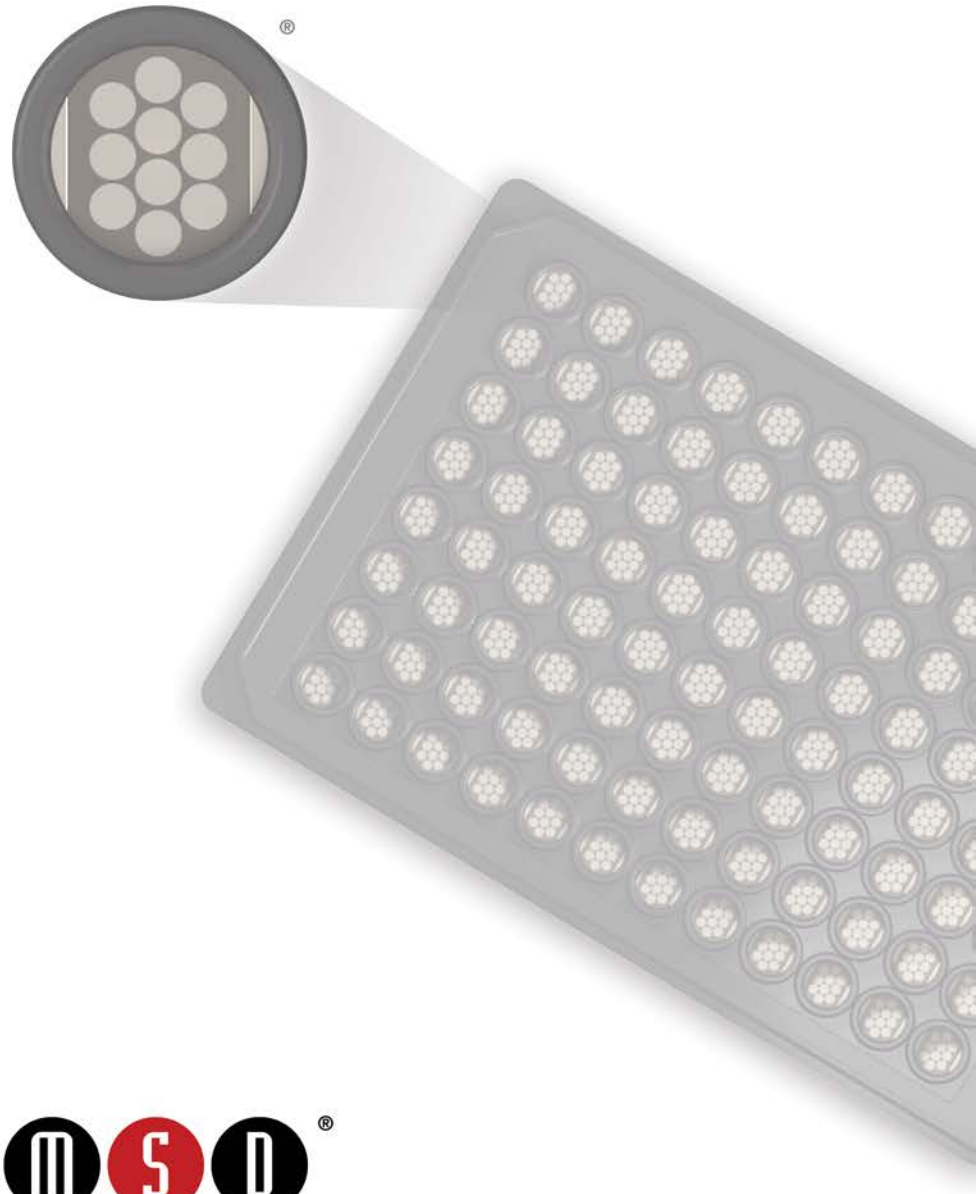
MSD® MULTI-SPOT Assay System

Proinflammatory Panel 1 (rat) Kits

IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, KC/GRO, IL-10, IL-13, TNF-α

V-PLEX®

SECTOR™ Assay Kits



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MSD Cytokine Assays

Proinflammatory Panel 1 (rat) Kits

IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, KC/GRO, IL-10, IL-13, TNF-α

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

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

MSD offers V-PLEX® assays for customers who require unsurpassed performance and quality. V-PLEX products are developed under rigorous design control and are fully validated according to fit-for-purpose principles following MSD's Quality Management System. They offer exceptional sensitivity, simple protocols, reproducible results, and lot-to-lot consistency. In addition to analytical validation, the 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 on 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.

The Proinflammatory Panel 1 (rat) measures ten cytokines that are important in inflammation, immune system regulation, and numerous other biological processes. These secreted biomarkers can be detected in a variety of tissues and bodily fluids and their over- or under-expression may indicate a shift in biological equilibrium of the body. This panel also consists of many of the Th1/Th2 pathway biomarkers. The Proinflammatory Panel 1 (rat) measures biomarkers that are implicated in a number of disorders including rheumatoid arthritis,[1] Alzheimer's disease,[2] asthma,[3] atherosclerosis,[4] allergies,[5] systematic lupus erythematosus,[6] obesity,[7] cancer,[8] depression,[9] multiple sclerosis,[10] diabetes,[11] psoriasis,[12] and Crohn's disease[13]. Because of their association with such a wide spectrum of disease, these biomarkers are the focus of drug discovery efforts and basic research.

The Proinflammatory Panel 1 includes the next generation of multiple rat cytokine assays that are part of the V-PLEX Proinflammatory Panel 2 (rat) products, in addition to a new rat IL-2 assay. The IL-13 and TNF-α antibody generations are unchanged. The biomarkers constituting the panel are: IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, KC/GRO, IL-10, IL-13, and TNF-α.

Principle of the Assay

MESO SCALE DISCOVERY® assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The assays in the Proinflammatory Panel 1 (rat) kits 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 IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, KC/GRO, IL-10, IL-13, and TNF-α assays are provided on 10-spot MULTI-SPOT plates (Figure 1). The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD GOLD™ SULFO-TAG) throughout one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds MSD GOLD Read Buffer B that creates the appropriate chemical environment for electrochemiluminescence (ECL) 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.[35]

1. IFN-γ
2. IL-1β
3. IL-2
4. IL-4
5. IL-5
6. IL-6
7. KC/GRO
8. IL-10
9. IL-13
10. TNF-α

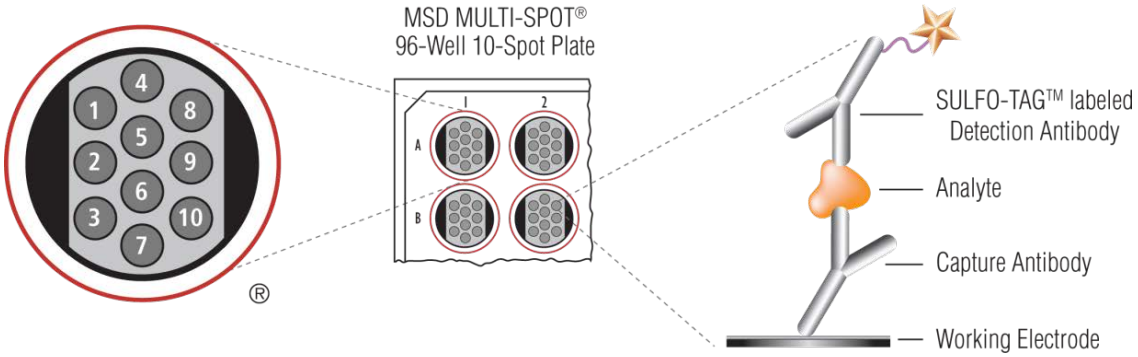


Figure 1. Multiplex plate spot diagram showing the placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Materials and Equipment

Kit Components

Proinflammatory Panel 1 (rat) assays are available as multiplex, individual, and 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.

See the Catalog Numbers section for a complete list of kits.

Reagents Supplied with All Kits

Table 1. Reagents that are supplied with V-PLEX and V-PLEX Plus Proinflammatory Panel 1 (rat) Kits

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1 Plate	5 Plates	25 Plates	
Proinflammatory Panel 1 (rat) Calibrator Blend	2–8 °C	C0294-2	1 vial	1 vial	5 vials	25 vials	Lyophilized kit calibrator
EDTA, 0.5 M pH 8.0	RT	—	1 vial	1 vial	1 vials	5 vials	Diuent additive to optimize assay performance
Diluent 65*	≤–10 °C	R50CJ-1	10 mL	1 bottle	—	—	Diluent for samples and calibrator
		R50CJ-2	50 mL	—	1 bottle	5 bottles	
Diluent 3*	≤–10 °C	R50AP-1	5 mL	1 bottle	—	—	Diluent for detection antibody
		R50AP-2	25 mL	—	1 bottle	5 bottles	
MSD GOLD Read Buffer B	RT	R60AM-1	18 mL	1 bottle	—	—	Buffer to catalyze the electro-chemiluminescence reaction
		R60AM-2	90 mL	—	1 bottle	5 bottles	

*Diluent 65, 10 mL and Diluent 3, 8 mL are provided as Diluent Assembly 35 (Catalog No. R50CL-1) whereas Diluent 65, 50 mL and Diluent 3, 40 mL are provided as Diluent Assembly 36 (Catalog No. R50CM-1).
RT = room temperature
Dash (—) = not applicable

V-PLEX Plus Kits: Additional Components

Table 2. Additional components that are supplied with V-PLEX Plus Proinflammatory Panel 1 (rat) Kits

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1 Plate	5 Plates	25 Plates	
Proinflammatory Panel 1 (rat) Control 1*	2–8 °C	—	1 vial	1 vial	5 vials	25 vials	Multi-analyte controls
Proinflammatory Panel 1 (rat) Control 2*	2–8 °C		1 vial	1 vial	5 vials	25 vials	
Proinflammatory Panel 1 (rat) Control 3*	2–8 °C		1 vial	1 vial	5 vials	25 vials	
Wash Buffer (20X)	RT	R61AA-1	100 mL	1 bottle	1 bottle	5 bottles	20-fold concentrated plate wash buffer solution
Plate Seals	—	—	—	3	15	75	Adhesive seals for sealing plates during incubations

*Controls are provided as components in the Proinflammatory Panel 1 (rat) Control Pack (Catalog No. C4294-1, 5-plate size pack). Refer to the control pack insert for more details.
RT = room temperature
Dash (—) = not applicable

Kit-Specific Components

Table 3. Components that are supplied with specific kits

Plate	Storage	Catalog No.	Size	Quantity Supplied			Description
				1 Plate	5 Plates	25 Plates	
Proinflammatory Panel 1 (rat) 96-well 10-spot SECTOR Plate	2–8 °C	N05294A-1	10-spot	1	5	25	96-well plate, foil sealed, with desiccant

Table 4. Individual detection antibodies for each assay are supplied with specific kits

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1 Plate	5 Plates	25 Plates	
Rat IFN-γ Antibody	2–8 °C	D23J2-2	75 µL	1	—	—	SULFO-TAG conjugated antibody
		D23J2-3	375 µL	—	1	5	
Rat IL-1 β Antibody	2–8 °C	D23K2-2	75 µL	1	—	—	SULFO-TAG conjugated antibody
		D23K2-3	375 µL	—	1	5	
Rat IL-2 Antibody	2–8 °C	D23L2-2	75 µL	1	—	—	SULFO-TAG conjugated antibody
		D23L2-3	375 µL	—	1	5	
Rat IL-4 Antibody	2–8 °C	D23M2-2	75 µL	1	—	—	SULFO-TAG conjugated antibody
		D23M2-3	375 µL	—	1	5	
Rat IL-5 Antibody	2–8 °C	D23N2-2	75 µL	1	—	—	SULFO-TAG conjugated antibody
		D23N2-3	375 µL	—	1	5	
Rat IL-6 Antibody	2–8 °C	D23P2-2	75 µL	1	—	—	SULFO-TAG conjugated antibody
		D23P2-3	375 µL	—	1	5	
Rat KC/GRO Antibody	2–8 °C	D23Q2-2	75 µL	1	—	—	SULFO-TAG conjugated antibody
		D23Q2-3	375 µL	—	1	5	
Rat IL-10 Antibody	2–8 °C	D23R2-2	75 µL	1	—	—	SULFO-TAG conjugated antibody
		D23R2-3	375 µL	—	1	5	
Rat IL-13 Antibody	2–8 °C	D23OD-2	75 µL	1	—	—	SULFO-TAG conjugated antibody
		D23OD-3	375 µL	—	1	5	
Rat TNF- α Antibody	2–8 °C	D23QW-2	75 µL	1	—	—	SULFO-TAG conjugated antibody
		D23QW-3	375 µL	—	1	5	

Dash (—) = not applicable

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
- ❑ MSD Wash Buffer catalog no. R61AA-1 (included in V-PLEX Plus kits)
- ❑ Adhesive plate seals (3 per plate included in V-PLEX Plus kits)
- ❑ Deionized water
- ❑ Vortex mixer

Optional Materials

- ❑ Proinflammatory Panel 1 (rat) Control Pack, available for separate purchase from MSD, catalog no. C4294-1 (included in V-PLEX Plus kits)
- ❑ 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 applicable safety data sheet(s) (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

Protocol

Best Practices

- Mixing or substituting reagents from different sources or 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 22–25 °C water bath prior to use. Thaw other reagents on wet ice and use them immediately.
- 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 antibodies (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 the introduction of bubbles. For empty wells, pipette gently to the bottom corner. Do not touch the pipette tip to the bottom of the wells when pipetting into the MSD plate.
- Tap the plate on a paper towel to remove residual fluid after washing.
- If an incubation step needs to be extended, leave the sample or detection antibody solution in the plate to keep the plate from drying out.
- Remove the plate seal before reading the plate.
- Read buffer should be at room temperature (20–26 ºC) when 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 an 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 the first thaw, aliquot Diluent 65 and Diluent 3 into suitable volumes before refreezing.

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.

For one plate, combine:

- ❑ 15 mL of MSD Wash Buffer (20X)
- ❑ 285 mL of deionized water

Prepare Proinflammatory Working Solution

This is used for preparing the calibrator and controls, and for diluting samples.

For one plate, combine the following in a 15-mL tube:

- ❑ 6860 µL of Diluent 65
- ❑ 140 µL of EDTA

Prepare Calibrator Dilutions

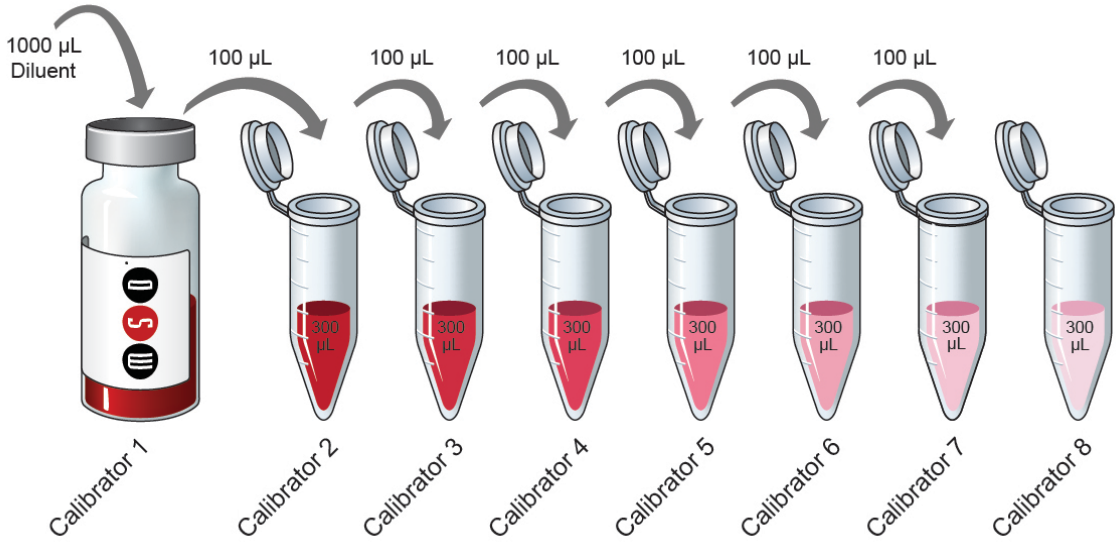
MSD supplies a multi-analyte lyophilized calibrator that yields the recommended highest calibrator concentration when reconstituted in 1,000 µL of Proinflammatory Working Solution. (For individual assays that do not saturate at the highest calibrator concentration, the calibration curve can be extended by creating a more concentrated highest calibrator. Follow the steps below using 250 µL instead of 1,000 µL of Proinflammatory Working Solution when reconstituting the lyophilized calibrator.)

To prepare 7 calibrator solutions plus a zero calibrator for up to 4 replicates (Figure 2):

1) Prepare the most concentrated calibrator (Calibrator 1) by adding 1,000 µL of Proinflammatory Working Solution 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 Calibrator 1 to 300 µL of Proinflammatory Working Solution. Mix well by vortexing. Repeat 4-fold serial dilutions 5 additional times to generate 7 calibrators.
3) Use Proinflammatory Working Solution as the zero calibrator.

Note: Reconstituted calibrator (Calibrator 1) is not stable when stored at 2–8 °C. It may be stored frozen at ≤-70 °C and is stable through five 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 2. *Dilution schema for preparation of Calibrator Standards.*



Sample Collection and Handling

Below are general guidelines for rat 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 before using or freezing. If no particulates are visible, you may not need to centrifuge.
- Other samples. Use immediately or freeze.

Freeze all samples in suitably-sized aliquots; they may be stored at ≤-70 ºC until needed. Repeated freeze-thaw of samples is not recommended. After thawing, centrifuge samples at 2,000g for 3 minutes to remove particulates before sample preparation.

Dilute Samples

Dilute samples with Proinflammatory Working Solution. For rat serum, plasma, and urine samples, MSD recommends a minimum 4-fold dilution. For example, when running samples in duplicate, add 50 µL of sample to 150 µL of Proinflammatory Working Solution. We recommend running at least two replicates per sample. When running unreplicated samples, add 25 µL of sample to 75 µL of Proinflammatory Working Solution. 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 Proinflammatory Panel 1 (rat) Control Pack, catalog no. C4294-1. (Controls are included only in V-PLEX Plus kits.)

Reconstitute the lyophilized controls in 250 µL of Proinflammatory Working Solution. Do not invert or vortex the vials. Wait for 15–30 minutes at room temperature before diluting controls 4-fold in Proinflammatory Working Solution. Vortex briefly using short pulses. For the lot-specific concentration of each analyte in the control, refer to the Proinflammatory Panel 1 (rat) Control Pack COA for analyte levels. Reconstituted controls must be stored frozen. They are stable through three freeze-thaw cycles.

Prepare MSD Plate

MSD V-PLEX 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. Prewash the plates before use as recommended in the assay protocol.

❖ PROTOCOL STEP 1: Wash and Add Sample

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

  Note: Washing the plate before 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 before sample addition.

Prepare Detection Antibody Solution

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

For one plate, combine the following detection antibodies and add to 2,400 µL of Diluent 3:

- ❑ 60 µL of SULFO-TAG Rat IFN-γ Antibody
- ❑ 60 µL of SULFO-TAG Rat IL-1β Antibody
- ❑ 60 µL of SULFO-TAG Rat IL-2 Antibody
- ❑ 60 µL of SULFO-TAG Rat IL-4 Antibody
- ❑ 60 µL of SULFO-TAG Rat IL-5 Antibody
- ❑ 60 µL of SULFO-TAG Rat IL-6 Antibody
- ❑ 60 µL of SULFO-TAG Rat KC/GRO Antibody
- ❑ 60 µL of SULFO-TAG Rat IL-10 Antibody
- ❑ 60 µL of SULFO-TAG Rat IL-13 Antibody
- ❑ 60 µL of SULFO-TAG Rat TNF-α 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.

❖ PROTOCOL STEP 2: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ Add 25 µL/well 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.

Prepare MSD GOLD Read Buffer B

MSD provides MSD GOLD Read Buffer B ready for use. Do not dilute.

❖ PROTOCOL STEP 3: Wash and Read

- ❑ Wash the plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ Add 150 µL/well of MSD GOLD Read Buffer B to each well. Analyze the plate on an MSD instrument.

Assay 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 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.

STEP 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 2 hours.

STEP 3: Wash and Read

- ❑ Wash the plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ Add 150 µL of MSD GOLD Read Buffer B to each well. Analyze the plate on an MSD instrument.

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 °C 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 Proinflammatory Working Solution 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 similar to the recommended protocol (data not shown).

Assay Characteristics

Validation

V-PLEX products are validated according to fit-for-purpose principles[35] 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.

- 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 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 assessing all validation lots.

- Accuracy and Precision

  Accuracy and precision are evaluated by measuring calibrators and controls across multiple runs and lots. For most assays, the results of control measurements fall within 25% of the expected concentration for each run. Precision is reported as the coefficient of variation (CV). Intra-run CVs and inter-run CVs are below 25%. Rigorous management of inter-lot reagent consistency and calibrator production results in typical inter-lot CVs below 25%. 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 25%.

- Matrix Effects and Samples

  Matrix effects from serum, plasma, urine, and cell culture media are measured for development and validation. Dilution linearity and spike recovery studies are performed on individual samples rather than pooled samples to assess the variability of results due to matrix effects. The sample dilution suggested in the protocol gives an appropriate dilution factor for all assays in the multiplex. Depending on the samples, some assays may benefit from lower or higher dilution factors.

- **Specificity**

  The specificity of both capture and detection antibodies is measured during assay development. Antibody specificity is assessed by 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 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 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 30 days. Assay component (calibrator, antibody, control) stability was assessed through freeze-thaw testing and accelerated stability studies. The validation program includes a real-time stability study with scheduled performance evaluations of complete kits for up to 60 months from manufacture.

Representative data from the validation studies are presented in the following sections. The stock concentration of calibrator, measured limits of quantification, and specificity data for each lot can be found in the lot-specific COA 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 (3-4 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® and Methodical Mind® analysis software.

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

Typical Data

Data from the Proinflammatory Panel 1 (rat) kits were collected over three months of testing by multiple operators (55 runs in total). Calibration curve accuracy and precision were assessed for three kit lots. Representative data from one lot are presented below (Figure 3).The multiplex panel was tested with individual detection antibodies to demonstrate that the assays are independent. Appendix B compares results for each assay in the kit when the panel is run using the individual detection antibody versus all ten detection antibodies. The calibration curves were comparable. Calibration curves for each lot are presented in the lot-specific COA.

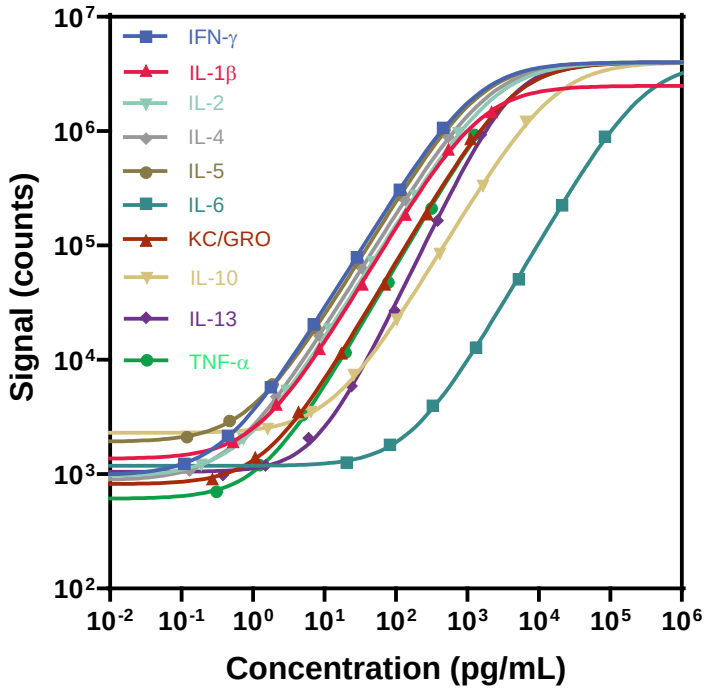


Figure 3. Typical calibration curves for assays in the V-PLEX Proinflammatory Panel 1 (rat) Kit.

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 55 runs.

The ULOQ is the highest concentration at which the CV of the calculated concentration is <25% and the recovery of each analyte is within 75% to 125% of the known value.

The LLOQ is the lowest concentration at which the CV of the calculated concentration is <25% and the recovery of each analyte is within 75% to 125% 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 included with each kit and available at www.mesoscale.com.

Table 5. LLOD, LLOQ, and ULOQ for each analyte in the Proinflammatory Panel 1 (rat) Kit

	Median LLOD (pg/mL)	LLOD Range (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
IFN-γ	0.03	0.02–0.37	1.21	292
IL-1β	0.11	0.05–0.36	2.66	1,230
IL-2	0.06	0.04–0.21	1.07	455
IL-4	0.08	0.03–1.26	3.62	357
IL-5	0.06	0.03–0.58	1.86	305
IL-6	13.06	6.85–57.87	188	54,600
KC/GRO	0.15	0.09–1.20	1.42	604
IL-10	1.80	0.33–17.13	43.1	3,900
IL-13	0.90	0.68–3.15	11.8	910
TNF-α	0.17	0.12–1.05	1.12	715

Precision

Precision was evaluated using the Proinflammatory Panel 1 (rat) Controls 1, 2, and 3. Analyte levels were measured by a minimum of three replicates on 48 runs over multiple days. The results are shown below. While a typical specification for precision is a concentration CV of less than 25% 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 variability of control replicates within an individual run, averaged over 48 runs.

Inter-run %CV is the variability of controls across 48 runs.

Inter-lot %CV is the variability of controls across three kit lots (48 runs).

Table 6. Intra-run and inter-run %CVs for each analyte in the Proinflammatory Panel 1 (rat) Kit

	Control	Average Conc. (pg/mL)	Average Intra-run %CV	Inter-run %CV	Inter-lot %CV
IFN-γ	Control 1	368	2.1	5.0	3.2
	Control 2	70	1.7	6.8	2.7
	Control 3	13	2.1	9.6	3.0
IL-1β	Control 1	915	1.9	14.6	5.0
	Control 2	104	2.1	15.3	6.7
	Control 3	15	3.7	16.9	8.9
IL-2	Control 1	635	2.0	4.8	3.6
	Control 2	91	2.1	7.0	3.0
	Control 3	13	2.8	9.9	4.0
IL-4	Control 1	465	4.3	8.4	6.5
	Control 2	150	4.4	8.4	5.6
	Control 3	43	3.9	10.1	4.9
IL-5	Control 1	415	1.7	4.5	0.6
	Control 2	98	1.7	14.8	4.0
	Control 3	22	2.6	22.7	11.2
IL-6	Control 1	67,886	1.6	4.2	0.2
	Control 2	9,467	1.7	7.5	1.1
	Control 3	1,774	1.8	10.1	3.5
KC/GRO	Control 1	875	2.6	5.2	2.5
	Control 2	130	2.9	6.6	0.3
	Control 3	20	3.3	9.6	4.8
IL-10	Control 1	5,798	2.8	6.6	2.7
	Control 2	1,904	1.4	6.4	2.6
	Control 3	557	1.1	7.9	2.1
IL-13	Control 1	1,389	1.9	6.2	6.3
	Control 2	423	2.1	6.6	6.0
	Control 3	151	1.4	6.2	4.9
TNF-α	Control 1	1,083	2.0	3.3	1.3
	Control 2	120	1.6	6.6	0.9
	Control 3	15	2.5	9.6	2.9

Tested Samples

Normal Samples

Normal rat serum, EDTA plasma, heparin plasma, citrate plasma, and urine samples, along with stimulated splenocyte cell culture supernatants, from a commercial source were diluted 4-fold and tested. Results for each sample set are displayed below. Concentrations are corrected for sample dilution. The median and range are calculated from samples with concentrations at or above the LLOD. The percentage detected is the percentage of samples with concentrations at or above the LLOD.

Table 7. Normal rat samples tested in the Proinflammatory Panel 1 (rat) Kit

Sample Type	Statistic	IFN-γ	IL-1β	IL-2	IL-4	IL-5	IL-6	KC/GRO	IL-10	IL-13	TNF-α
Serum (N=10)	Median (pg/mL)	2.85	1.87	1.27	0.703	1.14	136	224	13.4	22.2	6.22
	Range (pg/mL)	1.59–8.52	0.792–9.40	0.889–6.50	NA	0.420–3.99	120–1060	163–258	10.8–87.9	11.7–32.7	3.28–33.1
	% Detected	80	90	90	10	90	40	100	50	20	100
EDTA Plasma (N=10)	Median (pg/mL)	4.52	6.79	3.64	2.29	3.22	833	35.4	52.6	36.2	7.36
	Range (pg/mL)	0.990–7.41	1.77–11.8	0.442–9.44	1.61–8.51	0.870–6.66	428–1440	22.7–79.6	14.4–108	32.7–44.1	4.96–17.8
	% Detected	100	70	90	50	70	60	100	60	50	100
Heparin Plasma (N=10)	Median (pg/mL)	5.55	2.37	1.58	1.01	3.29	511	99.8	37.7	14.8	10.9
	Range (pg/mL)	1.78–13.9	0.962–49.0	0.844–22.4	0.616–4.27	0.862–11.8	111–7910	81.5–159	9.52–387	4.72–210	6.10–14.8
	% Detected	100	100	100	30	100	70	100	80	80	100
Citrate Plasma (N=10)	Median (pg/mL)	4.16	1.60	1.08	2.55	1.50	141	24.8	19.7	10.8	7.54
	Range (pg/mL)	1.94–17.5	0.976–15.5	0.492–7.47	0.896–4.21	0.659–6.00	113–1660	14.4–52.6	8.07–88.7	8.00–73.3	5.09–12.8
	% Detected	100	100	100	20	90	30	100	100	40	100
Urine (N=10)	Median (pg/mL)	ND	ND	ND	ND	0.523	ND	34.2	ND	ND	2.24
	Range (pg/mL)	NA	NA	NA	NA	0.444–0.601	NA	2.47–107	NA	NA	0.652–5.18
	% Detected	0	0	0	0	20	0	100	0	0	50
Cell Culture Supernatants (N=10)	Median (pg/mL)	2.07	1.58	24.4	23.6	40.9	653	116	342	310	139
	Range (pg/mL)	0.163–ADL	1.13–4.19	4.37–242	0.301–173	1.38–148	106–915	1.89–1220	9.12–1670	3.90–627	3.31–311
	% Detected	90	60	100	60	50	100	100	100	60	100

ADL = above detection limit; ND = not detectable; NA = not available

Dilution Linearity

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

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

Table 8. Analyte percent recovery at various dilutions in each sample type in the Proinflammatory Panel 1 (rat) Kit

		IFN-γ		IL-1β		IL-2		IL-4		IL-5	
Sample Type	Fold Dilution	Average% Recovery	% Recovery Range	Average% Recovery	% Recovery Range	Average% Recovery	% Recovery Range	Average% Recovery	% Recovery Range	Average% Recovery	% Recovery Range
Serum (N=6)	4	100	NA	100	NA	100	NA	100	NA	100	NA
	8	106	104-109	123	112-139	104	99-108	109	106-112	106	103-109
	16	108	105-114	154	140-170	109	105-113	113	105-123	107	101-111
	32	111	107-120	175	159-205	107	99-118	116	106-137	109	103-118
EDTA Plasma (N=6)	4	100	NA	100	NA	100	NA	100	NA	100	NA
	8	108	106-111	126	121-138	106	101-112	109	104-113	107	101-114
	16	109	102-115	153	141-171	108	100-116	109	96-126	107	101-116
	32	112	106-122	170	149-186	110	102-118	110	94-127	111	102-123
Heparin Plasma (N=6)	4	100	NA	100	NA	100	NA	100	NA	100	NA
	8	111	107-123	122	117-126	108	101-119	111	104-131	108	102-119
	16	115	106-136	152	144-160	110	101-119	114	105-142	109	100-118
	32	116	109-135	174	161-197	107	100-113	120	107-141	114	103-122
Citrate Plasma (N=6)	4	100	NA	100	NA	100	NA	100	NA	100	NA
	8	106	102-109	122	112-129	103	100-104	101	98-105	105	102-108
	16	107	105-108	160	150-176	106	104-108	103	95-110	107	105-109
	32	107	102-111	174	160-188	103	99-107	102	95-113	106	101-112
Urine (N=6)	4	100	NA	100	NA	100	NA	100	NA	100	NA
	8	109	103-127	110	102-129	102	95-118	109	99-132	104	98-121
	16	108	102-116	109	97-130	101	95-108	116	105-129	102	96-110
	32	109	102-115	109	99-128	98	93-105	122	102-137	104	100-108
Cell Culture Media (N=6)	4	100	NA	100	NA	100	NA	100	NA	100	NA
	8	103	101-106	102	91-112	102	99-107	107	98-112	102	96-107
	16	105	103-107	100	88-119	100	95-105	110	96-127	104	98-110
	32	107	103-113	101	90-120	100	97-107	117	100-140	105	99-112

NA = not available

Table 8. continued

		IL-6		KC/GRO		IL-10		IL-13		TNF-α	
Sample Type	Fold Dilution	Average% Recovery	% Recovery Range	Average% Recovery	% Recovery Range	Average% Recovery	% Recovery Range	Average% Recovery	% Recovery Range	Average% Recovery	% Recovery Range
Serum (N=6)	4	100	NA	100	NA	100	NA	100	NA	100	NA
	8	109	103-118	97	95-103	106	100-109	109	104-112	104	102-106
	16	123	108-137	104	97-109	109	102-114	122	115-127	106	101-110
	32	133	113-151	97	83-109	113	105-120	136	133-139	110	104-119
EDTA Plasma (N=6)	4	100	NA	100	NA	100	NA	100	NA	100	NA
	8	109	100-121	107	88-125	111	107-118	112	102-121	106	102-110
	16	116	104-138	112	91-134	113	107-119	125	109-137	108	96-116
	32	121	108-145	107	84-136	120	113-127	139	122-154	112	100-123
Heparin Plasma (N=6)	4	100	NA	100	NA	100	NA	100	NA	100	NA
	8	113	105-123	118	106-145	112	106-116	118	106-149	109	104-111
	16	117	109-132	128	106-167	117	106-132	131	116-155	111	104-122
	32	124	92-151	123	93-155	120	105-140	145	128-157	113	107-128
Citrate Plasma (N=6)	4	100	NA	100	NA	100	NA	100	NA	100	NA
	8	110	104-118	96	87-109	113	109-118	107	104-112	109	103-118
	16	121	114-126	105	90-121	120	108-130	119	112-129	114	102-131
	32	125	116-132	94	72-119	125	116-135	129	118-141	115	103-136
Urine (N=6)	4	100	NA	100	NA	100	NA	100	NA	100	NA
	8	106	98-122	102	88-121	104	98-120	109	99-133	102	97-117
	16	105	95-113	102	90-115	99	91-104	112	98-127	101	95-108
	32	104	95-112	94	76-109	95	90-99	122	103-143	99	95-101
Cell Culture Media (N=6)	4	100	NA	100	NA	100	NA	100	NA	100	NA
	8	97	94-100	100	92-108	105	99-110	95	92-100	98	96-99
	16	95	92-100	103	91-117	105	100-114	96	92-103	96	92-98
	32	95	90-102	101	89-115	109	103-122	102	96-115	97	93-102

NA = not available

Spike Recovery

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

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

Table 9. Spike recovery measurements of different sample types in the Proinflammatory Panel 1 (rat) Kit

	Serum (N=6)			EDTA Plasma (N=6)			Heparin Plasma (N=6)		
	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range
IFN-γ	100	3.8	93-105	99	2.6	95-105	106	3.5	101-113
IL-1β	59	4.5	51-68	67	5.8	60-80	61	7.9	51-83
IL-2	98	3.0	92-104	99	3.6	94-106	104	2.3	99-108
IL-4	105	7.0	91-115	116	7.0	101-126	112	5.9	105-123
IL-5	101	2.8	96-104	103	3.4	97-110	107	2.8	99-110
IL-6	72	8.6	55-85	85	11.3	64-101	80	12.7	65-114
KC/GRO	99	6.8	90-109	112	11.9	91-136	94	11.0	73-116
IL-10	95	3.9	88-100	95	3.5	86-101	90	7.1	76-99
IL-13	72	5.9	63-81	71	8.9	55-88	65	7.8	53-82
TNF-α	91	2.2	87-94	95	3.6	90-101	97	4.9	89-106

	Citrate Plasma (N=6)			Urine (N=6)			Cell Culture Media (N=6)		
	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range
IFN-γ	104	3.6	98-109	107	3.8	99-112	110	4.3	103-116
IL-1β	65	8.1	53-84	101	9.4	80-116	112	8.2	94-124
IL-2	105	3.3	99-110	108	2.3	104-114	108	3.3	102-113
IL-4	121	7.8	103-130	112	6.5	94-123	119	5.3	111-127
IL-5	106	5.1	97-114	112	3.0	107-117	110	4.8	100-116
IL-6	82	5.0	74-94	106	6.2	93-115	117	3.9	110-124
KC/GRO	107	9.6	87-119	111	7.3	97-126	113	5.5	103-122
IL-10	96	4.6	85-101	103	3.8	92-107	106	4.9	95-113
IL-13	76	8.8	62-94	94	9.2	74-111	112	4.6	103-118
TNF-α	90	10.0	73-100	102	2.7	95-107	108	3.8	102-115

Specificity

To access specificity, each assay in the panel was tested individually. Nonspecific binding was less than 1% for all assays in the kit. Non-specificity reported in the COA for this panel is measured using blended calibrators and individual detection antibodies.

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

Stability

The reconstituted calibrator, controls, and diluents were tested for freeze-thaw stability. Results (not shown) demonstrated that the reconstituted calibrator, Diluent 65, and Diluent 3 can go through five freeze-thaw cycles without significantly affecting the assay's performance. The reconstituted controls can go through three freeze-thaw cycles. The validation study includes an on-going real-time stability study with scheduled performance evaluations of complete kits for up to 60 months from date of manufacture.

Calibration

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

Assay Components

Calibrators

The assay calibrator blend uses the following recombinant rat proteins:

Table 10. Recombinant rat proteins used in the calibrators

	Expression System
IFN-γ	*E. coli*
IL-1β	*E. coli*
IL-2	*E. coli*
IL-4	*E. coli*
IL-5	*E. coli*
IL-6	*E. coli*
KC/GRO	*E. coli*
IL-10	*E. coli*
IL-13	*E. coli*
TNF-α	*E. coli*

Antibodies

Table 11. Antibody source species

	Source Species		
	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
IFN-γ	Mouse Monoclonal	Mouse Monoclonal	B
IL-1β	Mouse Monoclonal	Mouse Monoclonal	B
IL-2	Mouse Monoclonal	Mouse Monoclonal	B
IL-4	Mouse Monoclonal	Mouse Monoclonal	B
IL-5	Mouse Monoclonal	Mouse Monoclonal	B
IL-6	Mouse Monoclonal	Mouse Monoclonal	B
KC/GRO	Mouse Monoclonal	Mouse Monoclonal	B
IL-10	Mouse Monoclonal	Mouse Monoclonal	B
IL-13	Mouse Monoclonal	Goat Polyclonal	B
TNF-α	Hamster Monoclonal	Goat Polyclonal	A

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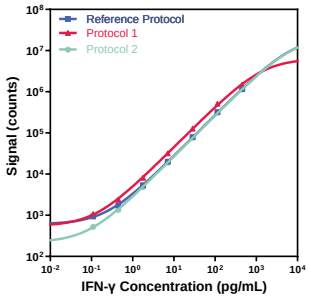
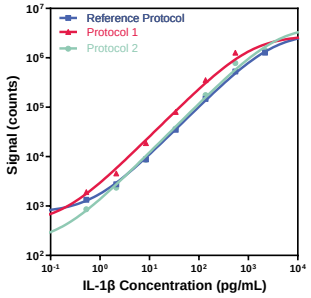
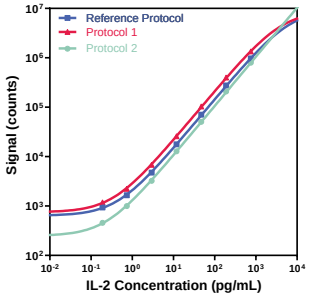
Additional Information

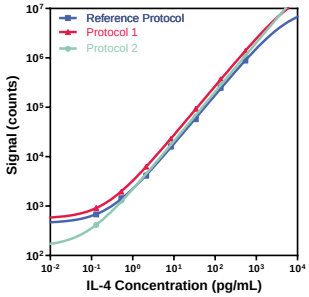
Appendix A: Relative Sensitivity under Alternate Protocols

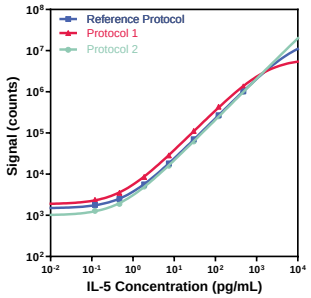
The calibration curves below illustrate the relative sensitivity of each assay under **Alternate Protocols**: Reference Protocol (2-hour sample incubation/2 wash steps, blue curve), Alternate Protocol 1 (overnight sample incubation, red curve), and Alternate Protocol 2 (tissue culture: single wash, green curve).

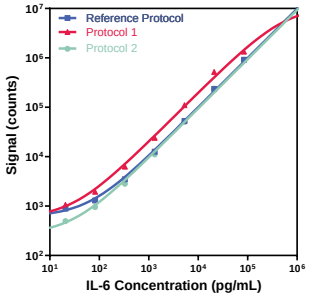


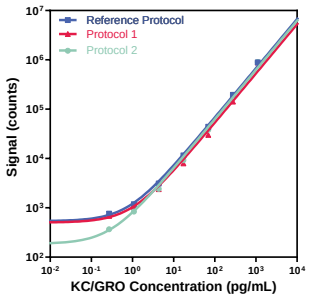


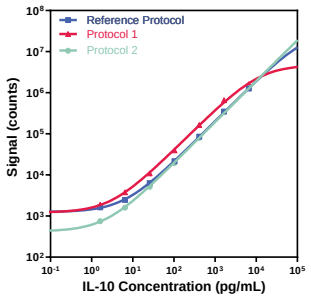


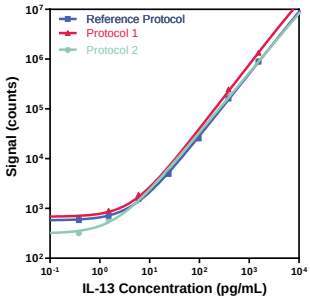


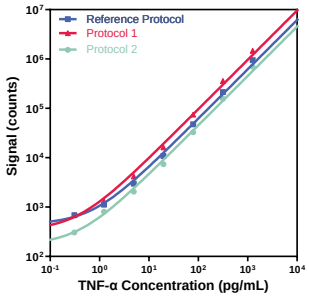










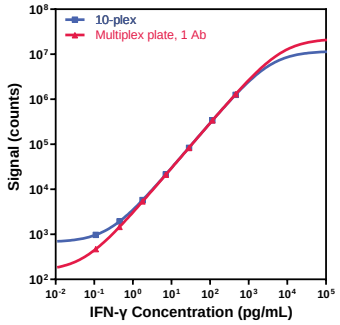


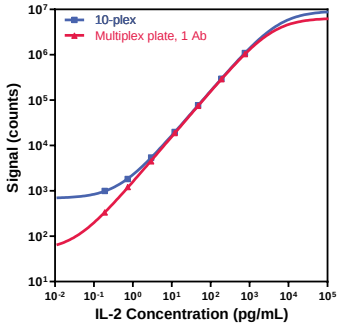
	LLOD Comparison (pg/mL)		
	Reference Protocol	Protocol 1	Protocol 2
IFN-γ	0.041	0.036	0.031
IL-1β	0.101	0.100	0.114
IL-2	0.061	0.040	0.080
IL-4	0.044	0.083	0.044
IL-5	0.062	0.055	0.073
IL-6	14.9	6.93	12.7
KC/GRO	0.154	0.273	0.189
IL-10	0.707	0.262	0.452
IL-13	0.869	0.764	0.591
TNF-α	0.169	0.133	0.230

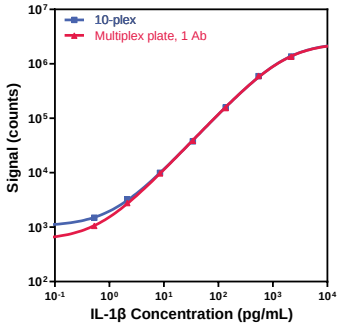
Table12. *Relative sensitivity when using alternate protocols*

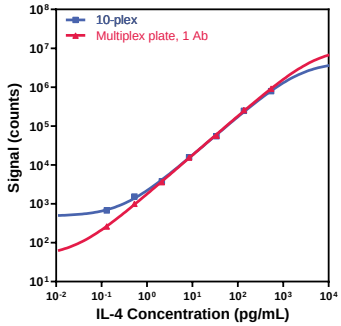
Appendix B: All vs Single Antibody

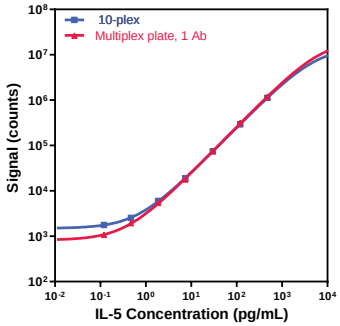
The calibration curves below compare results for each assay in the panel when the assays were run on the multiplex plate using all detection antibodies (blue curve) vs. running each assay using a single, assay-specific detection antibody (red curve).

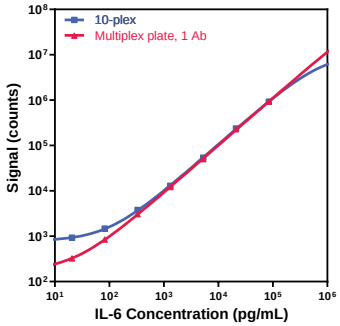


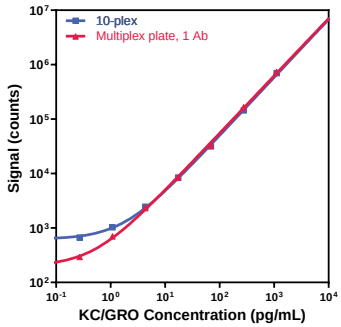


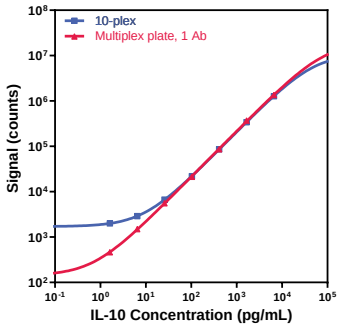


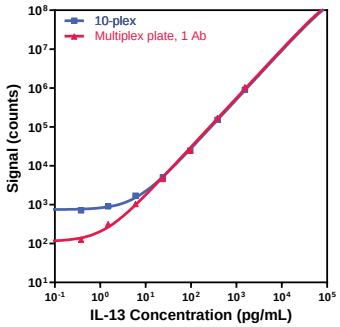


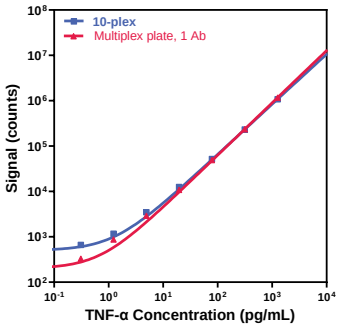












	LLOD (pg/mL)	
	Multiplex plate, 1 Ab	10-plex
IFN-γ	0.027	0.043
IL-1β	0.082	0.092
IL-2	0.052	0.055
IL-4	0.048	0.057
IL-5	0.033	0.110
IL-6	9.73	10.7
KC/GRO	0.187	0.556
IL-10	0.367	2.126
IL-13	0.726	1.905
TNF-α	0.175	0.789

Table13. Assay performance for individual and 10-plex assays

Summary Protocol

Proinflammatory Panel 1 (rat) Kits

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol before performing the Proinflammatory Panel 1 (rat) assays.

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature.
- ❑ Prepare calibration solutions in Proinflammatory Working Solution 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 samples and controls 4-fold in Proinflammatory Working Solution before adding to the plate.
- ❑ Prepare combined detection antibody solution by diluting each 50X detection antibody 50-fold in Diluent 3.

STEP 1: Wash and Add Sample

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

Catalog Numbers

Table 14. Catalog numbers for V-PLEX and V-PLEX Plus Proinflammatory Panel 1 (rat) multiplex and individual assays

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						
Proinflammatory Panel 1 (rat)	K15294D-1	K15294D-2	K15294D-4	K15294G-1	K15294G-2	K15294G-4
Individual SECTOR Assay Kits						
Rat IFN-γ v2	K153J2D-1	K153J2D-2	K153J2D-4	K153J2G-1	K153J2G-2	K153J2G-4
Rat IL-1β v2	K153K2D-1	K153K2D-2	K153K2D-4	K153K2G-1	K153K2G-2	K153K2G-4
Rat IL-2 v2	K153L2D-1	K153L2D-2	K153L2D-4	K153L2G-1	K153L2G-2	K153L2G-4
Rat IL-4 v2	K153M2D-1	K153M2D-2	K153M2D-4	K153M2G-1	K153M2G-2	K153M2G-4
Rat IL-5 v2	K153N2D-1	K153N2D-2	K153N2D-4	K153N2G-1	K153N2G-2	K153N2G-4
Rat IL-6 v2	K153P2D-1	K153P2D-2	K153P2D-4	K153P2G-1	K153P2G-2	K153P2G-4
Rat KC/GRO v2	K153Q2D-1	K153Q2D-2	K153Q2D-4	K153Q2G-1	K153Q2G-2	K153Q2G-4
Rat IL-10 v2	K153R2D-1	K153R2D-2	K153R2D-4	K153R2G-1	K153R2G-2	K153R2G-4
Rat IL-13 v2	K153S2D-1	K153S2D-2	K153S2D-4	K153S2G-1	K153S2G-2	K153S2G-4
Rat TNF-α v2	K153B2D-1	K153B2D-2	K153B2D-4	K153B2G-1	K153B2G-2	K153B2G-4

Plate Diagram

Figure 7. Plate diagram.