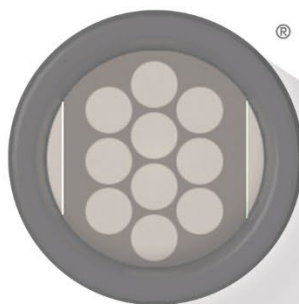


# MSD<sup>®</sup> MULTI-SPOT Assay System

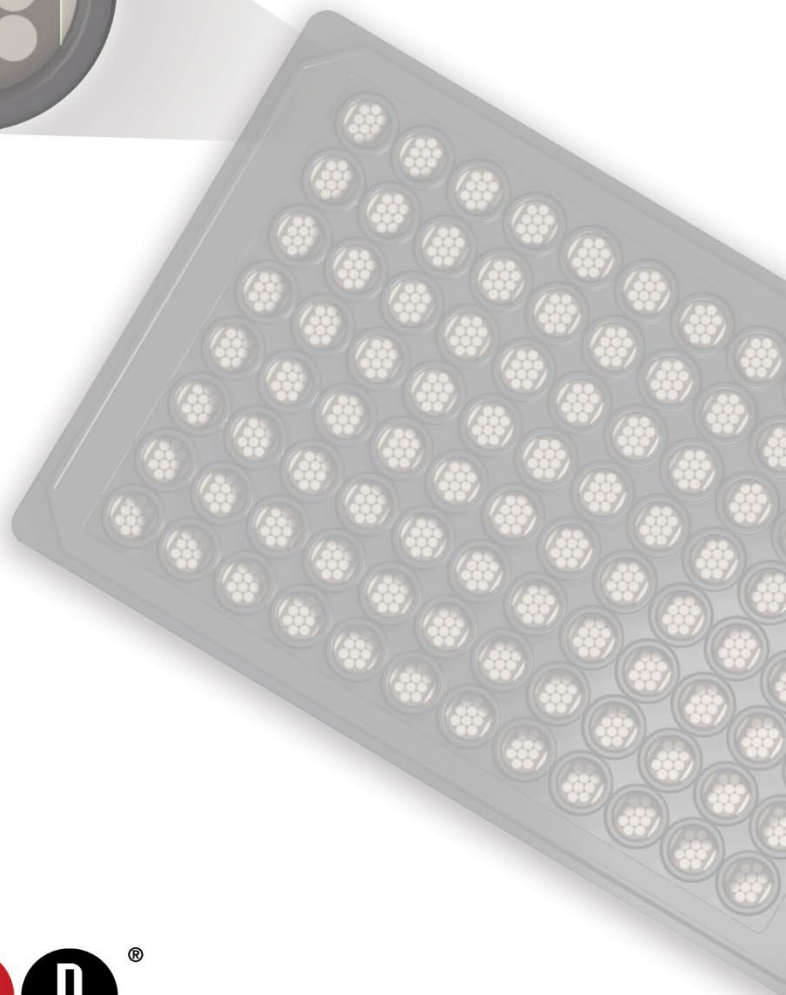
## V-PLEX<sup>®</sup> Immune Response Panel 1 (human) Kit

GM-CSF, IFN- $\gamma$ , IL-1 $\beta$ , IL-2, IL-6, IL-12p70, IL-10, IL-8, IL-17A, TNF- $\alpha$

**V**-PLEX<sup>®</sup>



**SECTOR<sup>™</sup> Assay Kits**  
**QuickPlex Ultra<sup>™</sup> Assay Kits**



[www.mesoscale.com](http://www.mesoscale.com)<sup>®</sup>

# MSD V-PLEX Platform

## V-PLEX Immune Response Panel 1 (human) Kit

GM-CSF, IFN- $\gamma$ , IL-1 $\beta$ , IL-2, IL-6, IL-12p70, IL-10, IL-8, IL-17A, TNF- $\alpha$

For use with human serum, plasma, and cell culture media.

### Instruments Supported:

SECTOR™ plates for use on MESO® SECTOR S 600, MESO SECTOR® S 600MM, MESO QuickPlex® SQ 120, and MESO QuickPlex SQ 120MM instrument

QuickPlex Ultra™ plates for use on MESO QuickPlex SQ 120, and MESO QuickPlex SQ 120MM instrument, MESO QuickPlex Q 60MM

*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

A division of Meso Scale Diagnostics, LLC.

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# Introduction

The MESO SCALE DISCOVERY® V-PLEX Immune Response Panel 1 (human) Kit measures Granulocyte-macrophage colony-stimulating factor (GM-CSF), Interferon gamma (IFN- $\gamma$ ), Interleukin-1 beta, (IL-1 $\beta$ ), Interleukin-2 (IL-2), Interleukin-6 (IL-6), Interleukin-12p70 (IL-12p70), Interleukin-10 (IL-10), Interleukin-8 (IL-8), Interleukin-17A (IL-17A), and Tumor necrosis factor alpha (TNF- $\alpha$ ). The markers of the V-PLEX Immune Response Panel 1 create a powerful multiplex for interrogating immune modulation including early indicators of inflammatory toxicity, T cell expansion, and cytotoxic T cell activity. These factors are produced following immune recognition and engagement of infected, stressed, or malignant target cells in both *in vitro* model systems and *in vivo* biological samples. The utility of this kit extends from autoimmune disorders to cancer therapeutics development.

This multiplex kit is validated using a simplified two-step protocol that delivers results in under 3 hours.

# Principle of the Assay

The V-PLEX Immune Response Panel 1 (human) Kit uses a sandwich immunoassay format on a MULTI-SPOT® 96-well plate, where individual capture antibodies are pre-coated directly onto spatially defined spots within each well (Figure 1).

During the assay, detection antibodies, samples, and calibrators are added to the plate simultaneously and allowed to incubate, forming sandwich complexes at each spot. After incubation and washing, MSD GOLD™ Read Buffer B, is added to the plate. The plate containing the sandwich assay is loaded onto an MSD instrument. The instrument automatically performs the electrochemiluminescence (ECL) reaction by applying an electric current to each well. The SULFO-TAG™ label undergoes electrochemical excitation and emits light at ~620 nm, which is detected and quantified by the instrument for each individual spot. Signal is measured by an MSD instrument, which images each well and quantifies light emission from each individual spot.

Signal intensity is proportional to the amount of analyte captured at each spot. Quantitative analyte concentrations in unknown samples are calculated by interpolation from a calibration curve.

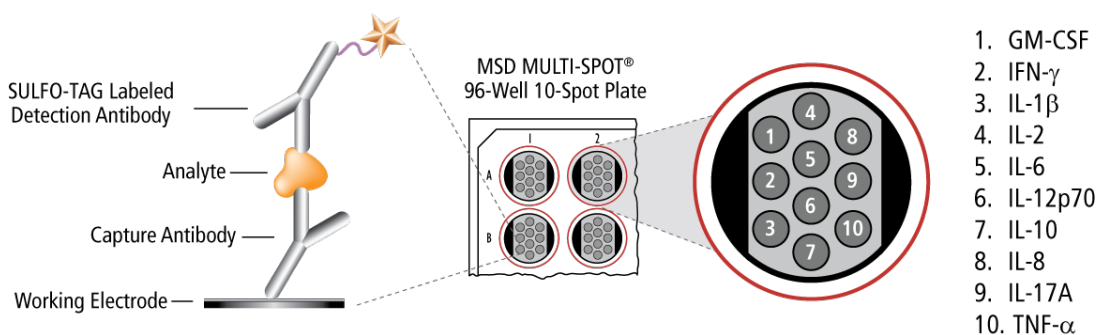


Figure 1. Multiplex plate spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. Custom combinations will be provided on 10-spot plates.

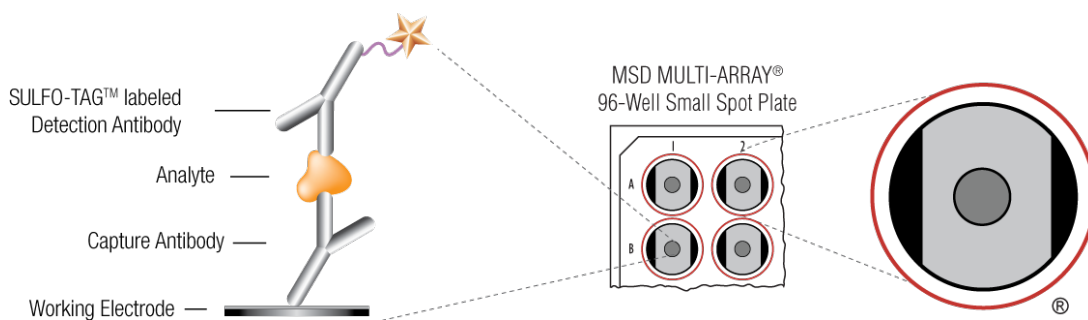


Figure 2. Small Spot plate diagram showing placement of analyte capture antibodies. Refer to Tables 1a and 1b for a list of assays provided on Small Spot plates.

# Kit Components

V-PLEX Immune Response Panel 1 (human) assays are available as a multiplex kit or as individual singleplex kits. V-PLEX Plus kits contain three control reagents in addition to the components in the V-PLEX kit. Kits include kit lot-specific (Table 1, Table 2) and non-kit lot-specific reagents (Table 4, Table 6). Lot-specific information for each assay can be found in the certificate of analysis (COA).

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

**Note:** Components will be packaged by storage conditions for ease of storage and shipping.

## Kit Lot-Specific Reagents and Components

*Table 1a. SECTOR components that are supplied with the V-PLEX Immune Response Panel 1 (human) Kit.*

Plates	Storage	Plate Type	Included Catalog #
Immune Response Panel 1 (hu) 96-Well 10-Spot SECTOR Plate	2–8 °C	10-spot	K151742D/G, K151AWCD/G, K151AWDD/G, K151AWGD/G
Cytolytic Panel 1 (human) 96-Well 10-Spot SECTOR Plate	2–8 °C	10-spot	K151AVAD/G
Human IFN- $\gamma$ V2 96-Well Small Spot SECTOR Plate	2–8 °C	Small spot	K151AVCD/G
Human IL-6 V2 96-Well Small Spot SECTOR	2–8 °C	Small spot	K151AVED/G
Human IL-8 V2 96-Well Small Spot SECTOR	2–8 °C	Small spot	K151AWFD/G
Human TNF- $\alpha$ V2 96-Well Small Spot SECTOR Plate	2–8 °C	Small spot	K151AVGD/G

Catalog numbers ending in D represent V-PLEX kits, G represent V-PLEX Plus kits.

*Table 1b. QuickPlex components that are supplied with the V-PLEX Immune Response Panel 1 (human) Kit.*

Plates	Storage	Plate Type	Included Catalog #
Immune Response Panel 1 (human) 96-Well 10-Spot QuickPlex Ultra Plate	2–8 °C	10-spot	K151742D/G, K151AWCD/G, K151AWDD/G, K151AWFD/G, K151AWGD/G
Cytolytic Panel 1 (human) 96-Well 10-Spot QuickPlex Ultra Plate	2–8 °C	10-spot	K151AVAD/G, K151AVCD/G, K151AVED/G, K151AVGD/G

Catalog numbers ending in D represent V-PLEX kits, G represent V-PLEX Plus kits.

*Table 1c. Calibrator and diluent that are supplied with the V-PLEX Immune Response Panel 1 (human) Kit.*

Reagent	Storage	Catalog #	Size	Quantity Supplied			Description
				1 Plate	5 Plates	25 Plates	
Immune Response Panel 1 (human) Calibrator Blend	$\leq -70$ °C	C0742-2	1 vial	1 vial	5 vials	25 vials	Multi-analyte calibrator
Diluent 58	$\leq -10$ °C	R50CA-1	10 mL	1 bottle	—	—	Diluent for samples, calibrator, detection antibodies, and controls
		R50CA-2	50 mL	—	1 bottle	5 bottles	

Dash (—) = not applicable.

**Table 2. Individual detection antibodies for each assay supplied with specific kits.**

SULFO-TAG Conjugated Detection Antibody	Storage	Cap color	Catalog #	Size	Quantity Supplied		
					1 Plate	5 Plates	25 Plates
Human GM-CSF Antibody	2–8 °C	●	D21AVA-2	75 µL	1	—	—
		●	D21AVA-3	375 µL	—	1	5
Human IFN-γ Antibody	2–8 °C	●	D21AVC-2	75 µL	1	—	—
		●	D21AVC-3	375 µL	—	1	5
Human IL-1β Antibody	2–8 °C	●	D21AWC-2	75 µL	1	—	—
		●	D21AWC-3	375 µL	—	1	5
Human IL-2 Antibody	2–8 °C	●	D21AVD-2	75 µL	1	—	—
		●	D21AVD-3	375 µL	—	1	5
Human IL-6 Antibody	2–8 °C	●	D21AVE-2	75 µL	1	—	—
		●	D21AVE-3	375 µL	—	1	5
Human IL-12p70 Antibody	2–8 °C	●	D21AWD-2	75 µL	1	—	—
		●	D21AWD-3	375 µL	—	1	5
Human IL-10 Antibody	2–8 °C	●	D21AWE-2	75 µL	1	—	—
		●	D21AWE-3	375 µL	—	1	5
Human IL-8 Antibody	2–8 °C	●	D21AWF-2	75 µL	1	—	—
		●	D21AWF-3	375 µL	—	1	5
Human IL-17A Antibody	2–8 °C	●	D21AWG-2	75 µL	1	—	—
		●	D21AWG-3	375 µL	—	1	5
Human TNF-α Antibody	2–8 °C	●	D21AVG-2	75 µL	1	—	—
		●	D21AVG-3	375 µL	—	1	5

Dash (—) = not applicable.

**Table 3. Antibody source.**

	Source Species		Assay Generation
	Capture Antibody	Detection Antibody	
GM-CSF	Recombinant Monoclonal	Recombinant Monoclonal	B
IFN-γ	Recombinant Monoclonal	Recombinant Monoclonal	D
IL-1β	Recombinant Monoclonal	Recombinant Monoclonal	D
IL-2	Recombinant Monoclonal	Recombinant Monoclonal	B
IL-6	Recombinant Monoclonal	Recombinant Monoclonal	D
IL-12p70	Recombinant Monoclonal	Recombinant Monoclonal	C
IL-10	Recombinant Monoclonal	Recombinant Monoclonal	B
IL-8	Recombinant Monoclonal	Recombinant Monoclonal	C
IL-17A	Recombinant Monoclonal	Recombinant Monoclonal	C
TNF-α	Recombinant Monoclonal	Recombinant Monoclonal	C

## Non-Kit Lot-Specific Reagents and Components

Table 4. Reagents that are supplied with V-PLEX and V-PLEX Plus Immune Response Panel 1 (human) Kits.

Reagent	Storage	Catalog #	Size	Quantity Supplied			Description
				1 Plate	5 Plates	25 Plates	
MSD GOLD Read Buffer B	RT	R60AM-1	18 mL	1 bottle	—	—	Buffer to catalyze the electrochemiluminescent reaction
		R60AM-2	90 mL	—	1 bottle	5 bottles	

Dash (—) = not applicable.

Table 5. Recombinant human proteins used in the calibrators.

	Expression System
GM-CSF	<i>E. coli</i>
IFN- $\gamma$	<i>E. coli</i>
IL-1 $\beta$	<i>E. coli</i>
IL-2	<i>E. coli</i>
IL-6	<i>E. coli</i>
IL-12p70	<i>Sf21</i>
IL-10	<i>Sf21</i>
IL-8	<i>E. coli</i>
IL-17A	<i>E. coli</i>
TNF- $\alpha$	<i>E. coli</i>

### Additional Components

Controls are provided as components in the Immune Response Panel 1 (human) Control Pack (Catalog No. C4742-1). The Immune Response Panel 1 (human) Control Pack consists of 3 levels of controls, each containing concentrations of human proteins that are detected by the V-PLEX and V-PLEX Plus Immune Response Panel 1 (human) kits. The controls span the linear range and are designed to test the analytical range of the assays. These controls assess reproducibility of assay performance, and precision CVs are expected to be less than 25%. The certificate of analysis contains the concentrations of the controls measured at MSD across three lots. Even with good laboratory practices, site-to-site differences may occur; therefore, to establish accuracy specifications, it is recommended that each lab should establish its own nominal values and acceptance range for the control concentrations.

Table 6. Additional components that are supplied with V-PLEX Plus Immune Response Panel 1 (human) Kits.

Reagent	Storage	Cap Color	Catalog #	Size	Quantity Supplied			Description
					1 Plate	5 Plates	25 Plates	
Immune Response Panel 1 (human) Control 1	$\leq -70$ °C	●	C4742-1	1 vial	1 vial	5 vials	25 vials	Multi-analyte controls
Immune Response Panel 1 (human) Control 2	$\leq -70$ °C	●		1 vial	1 vial	5 vials	25 vials	
Immune Response Panel 1 (human) Control 3	$\leq -70$ °C	●		1 vial	1 vial	5 vials	25 vials	
Wash Buffer	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

Dash (—) = not applicable.

# Additional Materials and Equipment

## Materials

- Appropriately sized tubes for reagent preparation
- Polypropylene microcentrifuge tubes for preparing dilutions
- Liquid-handling equipment for desired throughput, capable of dispensing 10 to 150  $\mu\text{L}$ /well into a 96-well microtiter plate
- 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
- OPTIONAL: Immune Response Panel 1 (human) Control Pack, available for separate purchase from MSD, catalog no. C4742-1 (included in V-PLEX Plus kits)

## Equipment

- Plate-washing equipment: automated plate washer or multichannel pipette
- Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm
- Vortex mixer
- Water bath
- OPTIONAL: Centrifuge for sample preparation

## Safety

Use safe laboratory practices: wear gloves, safety glasses, and lab coats when handling assay 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), which can be obtained from MSD Customer Service or at the [www.mesoscale.com](http://www.mesoscale.com)<sup>®</sup> website.

# Best Practices

Read this product insert before use, and follow the best practices:

## Reagent Preparation

<b>Do Not Mix Lots</b>	Do not mix components between boxes of multiplex V-PLEX panels. Lot information is provided in the lot-specific COA.
<b>Thaw Reagents</b>	Bring frozen diluents to room temperature in a 22–25 °C water bath. Thaw other reagents on wet ice and use them immediately. Mix well before use. Bring plates to room temperature before opening the packet.

## Reagent Handling

<b>Avoid Bubbles During Pipetting</b>	Avoid bubbles at each stage of reagent addition because they can lead to variable results. This is especially important when adding read buffer (just prior to reading the plate).
<b>Prepare in Polypropylene Microcentrifuge Tubes</b>	Prepare standards and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution, and mix by vortexing after each dilution.
<b>Avoid Prolonged Light Exposure</b>	Avoid prolonged exposure of the detection reagent (stock or diluted) to light. During the plate incubation steps, plates do not need to be shielded from light (except for direct sunlight).

## Plate Handling

<b>Plate Shaking Guidelines</b>	Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm for 96-well plates. Keep the shaking speed and shaker model consistent for long-term studies. Binding reactions may reach equilibrium sooner if shaken in the middle of this range (~700 rpm) or above.
<b>Washing Fluid Removal</b>	Tap the plate on a paper towel after washing to ensure the removal of residual fluid.
<b>Some Assays Are Temperature Sensitive</b>	V-PLEX assays were characterized at 20–26 °C. Assays run above or below that range may be negatively affected.

## Plate Reading

<b>Remove Plate Seal</b>	Remove the plate seal before reading the plate in the instrument.
<b>Do Not Shake Plate</b>	Do not shake the plate after adding read buffer.
<b>Room Temperature Read Buffer</b>	Read buffer should be at room temperature (20-26 °C) when adding it to the plate. Do NOT shake the read buffer bottle before use.
<b>Timing of Plate Reads</b>	Keep time intervals consistent between the addition of read buffer and reading the plate to improve interplate precision. Prepare an MSD instrument before adding read buffer.
<b>Results Above Curve</b>	If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay.

# Summary Protocol

## Immune Response Panel 1 (human) Kits

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

### Sample and Reagent Preparation

- Bring all reagents to room temperature.
- Prepare calibration solutions in Diluent 58 using the supplied calibrator.
  - Thaw the frozen calibrator blend, then dilute in Diluent 58.
  - Vortex briefly using short pulses.
  - Perform a series of 4-fold dilution steps and prepare a zero calibrator.
- Dilute samples and controls 2-fold in Diluent 58 before adding to the plate. Thaw controls before dilution.
- Prepare combined detection antibody solution by diluting each 50X detection antibody 50-fold in Diluent 58.

### STEP 1: Wash and Add Detection Antibody Solution and Sample

- Wash plate 3 times with at least 150  $\mu\text{L}$ /well of Wash Buffer.
- Add 25  $\mu\text{L}$ /well of detection antibody solution and 25  $\mu\text{L}$ /well of sample (calibrators, controls, or unknowns) to each well.
- Incubate at room temperature with shaking for 2 hours.

### STEP 2: Wash and Read Plate

- Wash plate 3 times with at least 150  $\mu\text{L}$ /well of Wash Buffer.
- Add 150  $\mu\text{L}$ /well of MSD GOLD Read Buffer B.
- Analyze plate on the MSD instrument.

# Recommended Protocol

Bring all reagents to room temperature and refer to the **Best Practices** section (above) before beginning the protocol.

**Important:** Upon the first thaw, aliquot Diluent 58 into suitable volumes before refreezing.

Reagents prepared at each step are sufficient for a one-plate experiment.

## STEP 1: PREPARE SAMPLES AND REAGENTS

### Prepare Wash Buffer

MSD provides 100 mL of Wash Buffer as a 20X stock solution. Dilute the stock solution to 1X before use.

- ❑ 15 mL  $\mu$ L MSD Wash Buffer (20X)
- ❑ 285 mL of deionized water

### Prepare Calibrator Dilutions

MSD supplies a multi-analyte calibrator that yields the recommended highest calibrator concentration when diluted 20-fold in Diluent 58. 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 that case, follow the steps below using a dilution factor  $<20$  when diluting the stock calibrator.

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

- ❑ Thaw the stock calibrator on wet ice for at least 30 minutes and keep on ice.
- ❑ Prepare the most concentrated calibrator (Calibrator 1) by adding 20  $\mu$ L of stock calibrator to 380  $\mu$ L of Diluent 58. Mix well by vortexing.
- ❑ Prepare the next calibrator by transferring 100  $\mu$ L of Calibrator 1 to 300  $\mu$ L of Diluent 58. Mix well by vortexing. Repeat 4-fold serial dilutions 5 additional times to generate 7 calibrators.
- ❑ Use Diluent 58 as the zero calibrator.

Note: Stock (20X) calibrator can be stored at 4°C for up to 7 days. It may also be stored at  $\leq 70^{\circ}\text{C}$  and is stable through five freeze thaw cycles. Diluted (1X) calibrator can be stored at 4°C for up to 1 day. Do not store calibrator at RT beyond the day of testing. 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](http://www.mesoscale.com).

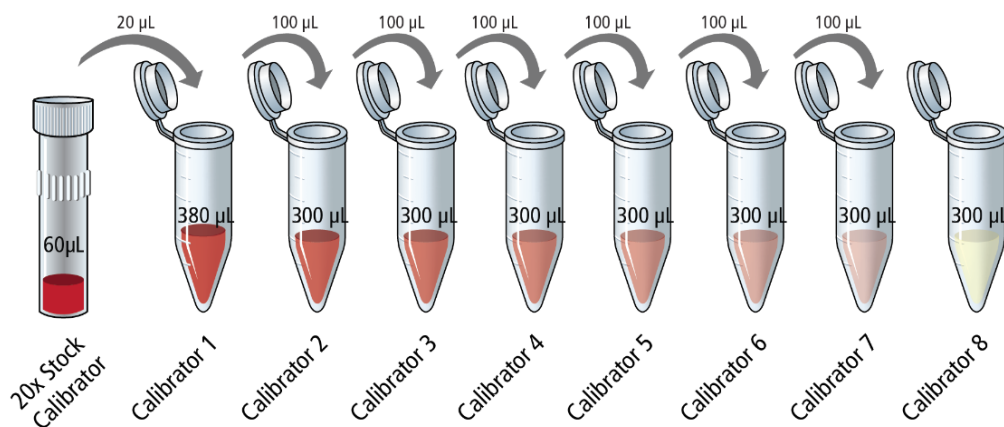


Figure 3. Dilution schema for preparation of Calibrator Standards.

## Dilute Samples

Dilute samples with Diluent 58. For human serum and plasma MSD recommends a minimum 2-fold dilution. For example, when running samples in duplicate, add 50  $\mu$ L of sample to 50  $\mu$ L of Diluent 58. We recommend running at least two replicates per sample. 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 duplicate. Additional diluent can be purchased at [www.mesoscale.com](http://www.mesoscale.com).

## Prepare Controls

Three levels of multi-analyte lyophilized controls are available for separate purchase from MSD in the Immune Response Panel 1 (human) Control Pack, catalog no. C4742-1. (Controls are included only in V-PLEX Plus kits.)

Thaw the controls on wet ice for at least 30 minutes. Dilute controls 2-fold in Diluent 58. Mix by vortexing.

Note: Stock (2X) controls are provided frozen and must be stored frozen and are stable through 5X freeze-thaw cycles. Stability of diluted (1X) controls has not been tested. For the lot-specific concentration of each analyte in the control, refer to the COA supplied with the control pack.

## 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  $\mu$ L of Diluent 58:

- 60  $\mu$ L of SULFO-TAG Human GM-CSF Antibody
- 60  $\mu$ L of SULFO-TAG Human IFN- $\gamma$  Antibody
- 60  $\mu$ L of SULFO-TAG Human IL-1 $\beta$  Antibody
- 60  $\mu$ L of SULFO-TAG Human IL-2 Antibody
- 60  $\mu$ L of SULFO-TAG Human IL-6 Antibody
- 60  $\mu$ L of SULFO-TAG Human IL-12p70 Antibody
- 60  $\mu$ L of SULFO-TAG Human IL-10 Antibody
- 60  $\mu$ L of SULFO-TAG Human IL-8 Antibody
- 60  $\mu$ L of SULFO-TAG Human IL-17A Antibody
- 60  $\mu$ L of SULFO-TAG Human TNF- $\alpha$  Antibody

For Individual assay kits, add 60  $\mu$ L of the supplied detection antibody to 2,940  $\mu$ L of Diluent 58.

## STEP 2: PREPARE MSD PLATE

### Wash and Add Detection Antibody Solution and Sample

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 plates before use as recommended in the assay protocol.

- Wash the plate 3 times with at least 150  $\mu\text{L}$ /well of Wash Buffer.
- Add 25  $\mu\text{L}$  of detection antibody solution to each well.
- Add 25  $\mu\text{L}$  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 before sample addition is an optional but recommended 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.

## STEP 3: WASH AND READ

### Wash and Add MSD GOLD Read Buffer B

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

Note: Diluting MSD GOLD Read Buffer B may compromise assay results.

- Wash the plate 3 times with at least 150  $\mu\text{L}$ /well of Wash Buffer.
- Add 150  $\mu\text{L}$  of MSD GOLD Read Buffer B to each well. Analyze the plate on an MSD instrument.

## Alternate Protocols

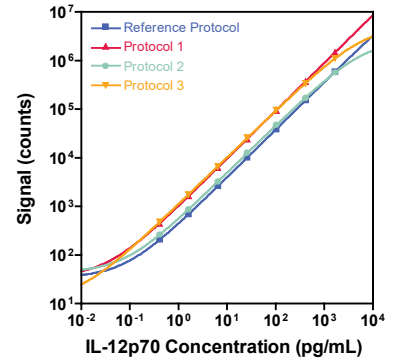
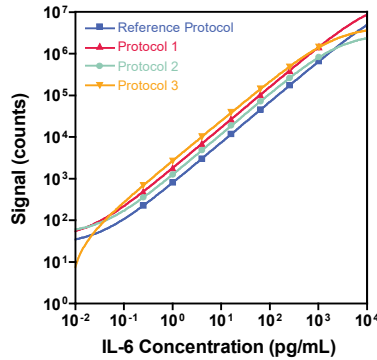
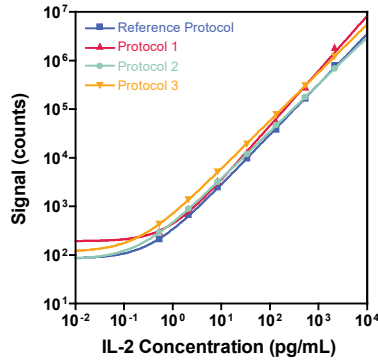
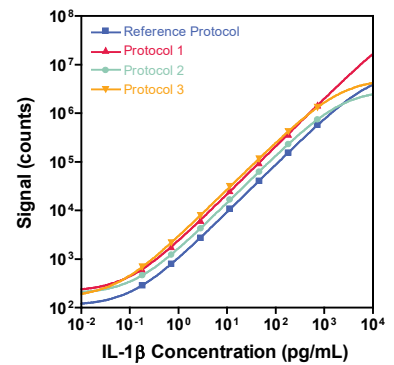
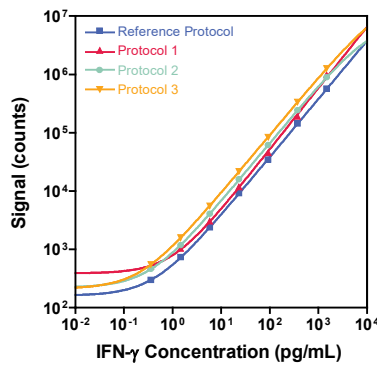
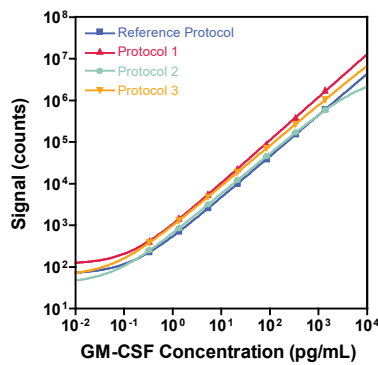
The suggestions below may be useful as alternate protocols; however, not all were tested using multiple kit lots.

- 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, Sequential Protocol: You may run the sequential V-PLEX protocol. Add 25  $\mu\text{L}$  of Diluent 58 to each well, then add 25  $\mu\text{L}$  of samples, calibrators and controls to the plate and incubate. After washing with 150  $\mu\text{L}$ /well of Wash Buffer, add 25  $\mu\text{L}$  of detection antibody solution (diluted 100X from stock) to each well. Proceed with the wash and read step. See Appendix A for assay performance using this protocol.
- Alternate Protocol 3, Extended Sample Incubation, Sequential Protocol: Incubating samples overnight at 2–8 °C may improve sensitivity for some assays. After washing with 150  $\mu\text{L}$ /well of Wash Buffer, add 25  $\mu\text{L}$  of detection antibody solution (diluted 100X from stock) to each well. Proceed with the wash and read step. See Appendix A for assay performance using this protocol.

# 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 and detect incubation, blue curve), Alternate Protocol 1 (overnight sample and detect incubation, red curve), Alternate Protocol 2 (2-hour sample incubation, wash, 2-hour detect incubation, green curve), and Alternate Protocol 3 (overnight sample incubation, wash, 2-hour detect incubation, yellow curve).



Graphs continued on next page

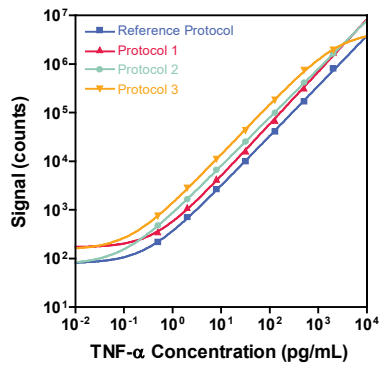
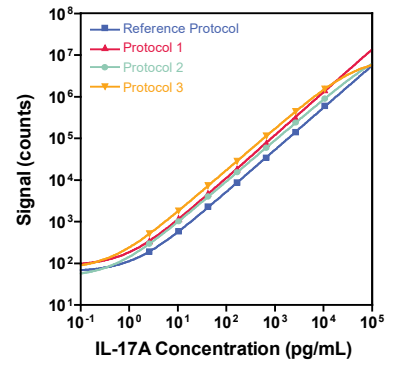
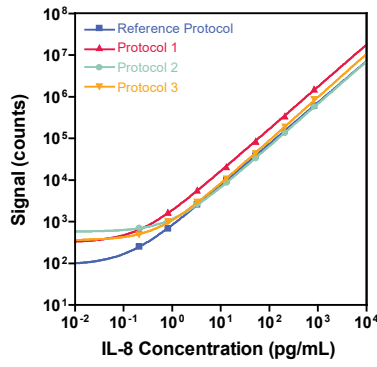
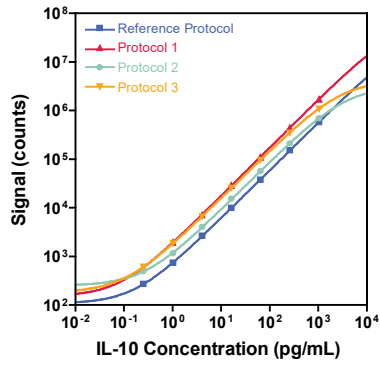
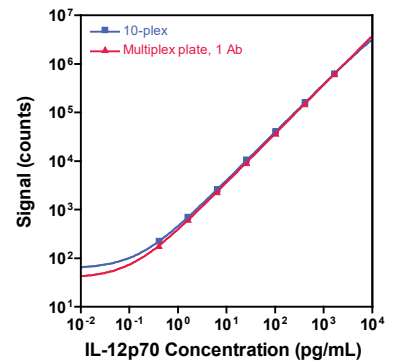
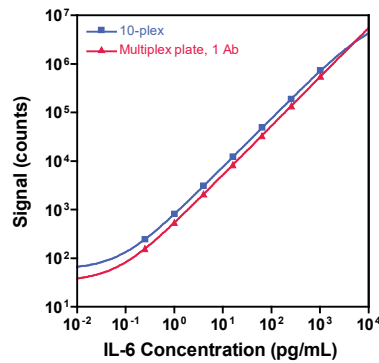
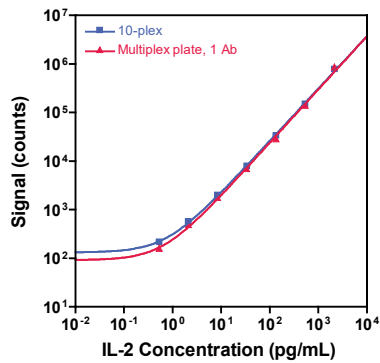
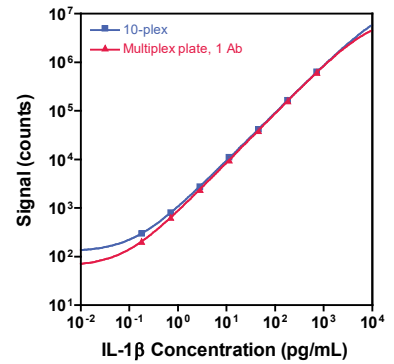
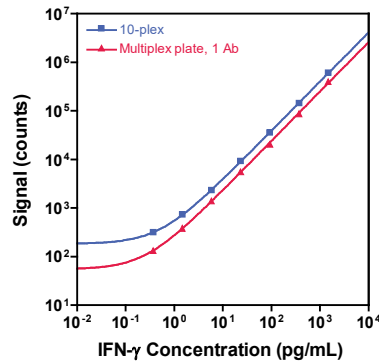
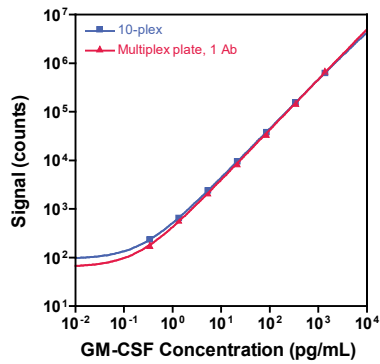


Table 7. Relative sensitivity when using alternate protocols

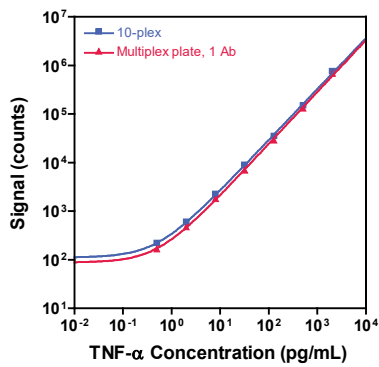
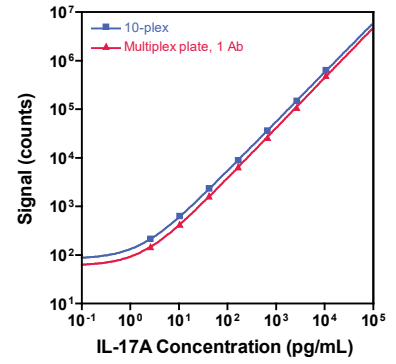
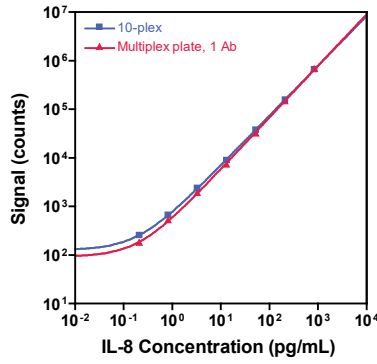
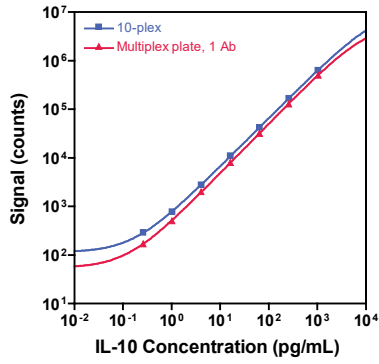
	LLOD Comparison (pg/mL)			
	Reference Protocol	Protocol 1	Protocol 2	Protocol 3
GM-CSF	0.149	0.080	0.124	0.076
IFN- $\gamma$	0.190	0.193	0.114	0.081
IL-1 $\beta$	0.073	0.033	0.050	0.026
IL-2	0.286	0.284	0.201	0.125
IL-6	0.099	0.044	0.063	0.028
IL-12p70	0.179	0.077	0.150	0.069
IL-10	0.110	0.044	0.078	0.044
IL-8	0.095	0.124	0.185	0.103
IL-17A	1.440	0.772	0.818	0.447
TNF- $\alpha$	0.237	0.176	0.095	0.061

## 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).



Graphs continued on next page

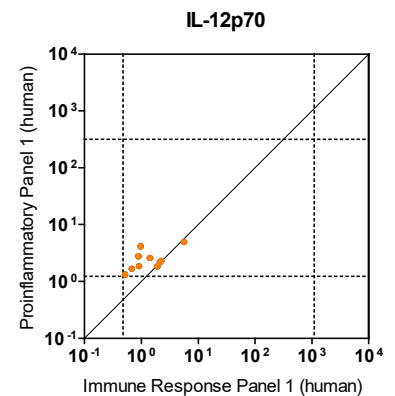
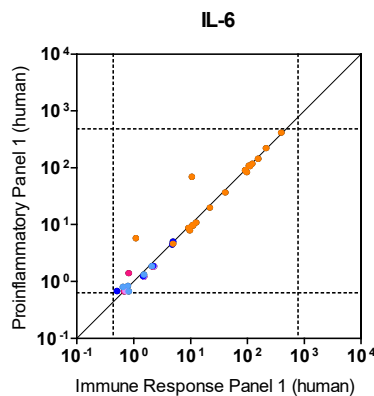
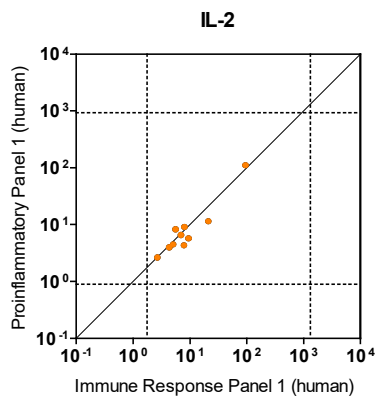
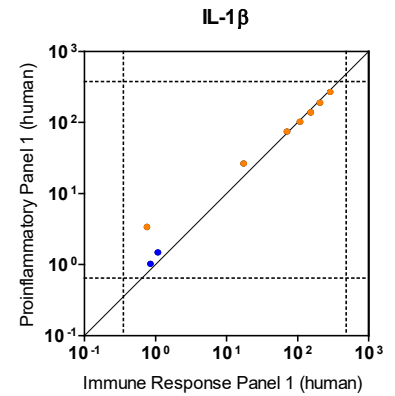
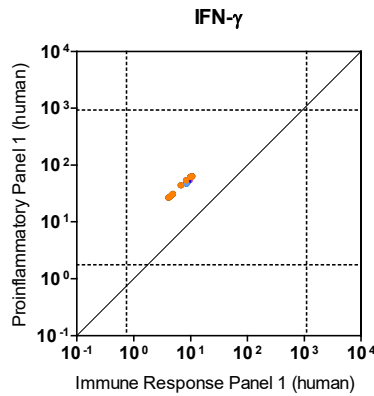
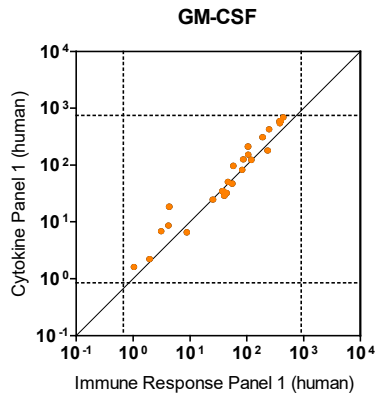


*Table 8. Assay performance for individual and 10-plex assays*

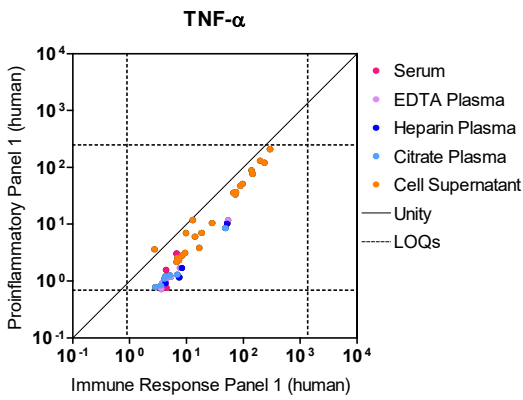
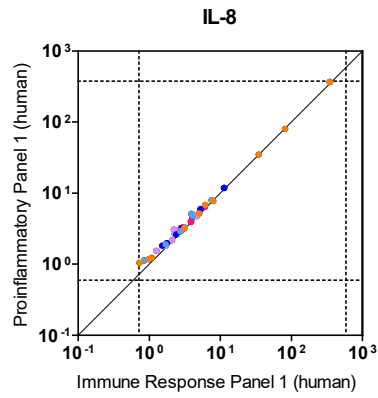
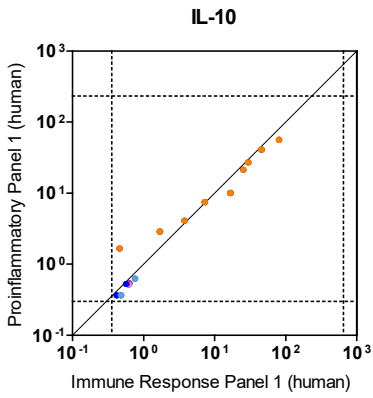
	LLOD (pg/mL)	
	Multiplex plate, 1 Ab	10-plex
GM-CSF	0.205	0.185
IFN- $\gamma$	0.323	0.209
IL-1 $\beta$	0.094	0.081
IL-2	0.493	0.402
IL-6	0.160	0.104
IL-12p70	0.215	0.197
IL-10	0.164	0.118
IL-8	0.165	0.121
IL-17A	2.120	1.480
TNF- $\alpha$	0.396	0.324

## Appendix C: Sample Concordance

Sample concordance was performed using commercially sourced serum, plasma, and cell culture supernatant samples between assays in the V-PLEX Immune Response Panel 1 (human) and V-PLEX Proinflammatory Panel 1 or V-PLEX Cytokine Panel 1 (human) Kits. Concordance was evaluated using slope (Deming regression) and  $r^2$  (Pearson correlation).



Graphs continued on next page



**Table 9.** Concordance between the assays in the V-PLEX Immune Response Panel 1 and existing V-PLEX assays.

	R <sup>2</sup>	Slope
GM-CSF	0.96	1.55
IFN-γ	0.98	0.63*
IL-1β	1.00	0.91
IL-2	0.98	1.17
IL-6	0.98	1.02
IL-12p70	0.46	0.67
IL-10	0.98	0.74
IL-8	1.00	1.05
IL-17A	0.98	1.44
TNF-α	0.96	0.62

\*NIBSC corrected

# Catalog Numbers

**Table 10.** Catalog numbers for V-PLEX and V-PLEX Plus Immune Response Panel 1 (human) 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
<b>Multiplex Kits</b>						
Immune Response Panel 1 (human)	K15742D-1	K15742D-2	K15742D-4	K15742G-1	K15742G-2	K15742G-4
Immune Response Panel 1 (human) QuickPlex	K15742D-21	K15742D-22	K15742D-24	K15742G-21	K15742G-22	K15742G-24
<b>Individual SECTOR Assay Kits</b>						
Human GM-CSF V2	K151AVAD-1	K151AVAD-2	K151AVAD-4	K151AVAG-1	K151AVAG-2	K151AVAG-4
Human IFN- $\gamma$ V2	K151AVCD-1	K151AVCD-2	K151AVCD-4	K151AVCG-1	K151AVCG-2	K151AVCG-4
Human IL-1 $\beta$ V2	K151AWCD-1	K151AWCD-2	K151AWCD-4	K151AWCG-1	K151AWCG-2	K151AWCG-4
Human IL-6 V2	K151AVED-1	K151AVED-2	K151AVED-4	K151AVEG-1	K151AVEG-2	K151AVEG-4
Human IL-12p70 V2	K151AWDD-1	K151AWDD-2	K151AWDD-4	K151AWDG-1	K151AWDG-2	K151AWDG-4
Human IL-8 V2	K151AWFD-1	K151AWFD-2	K151AWFD-4	K151AWFG-1	K151AWFG-2	K151AWFG-4
Human IL-17A V2	K151AWGD-1	K151AWGD-2	K151AWGD-4	K151AWGG-1	K151AWGG-2	K151AWGG-4
Human TNF- $\alpha$ V2	K151AVGD-1	K151AVGD-2	K151AVGD-4	K151AVGG-1	K151AVGG-2	K151AVGG-4
<b>Individual QuickPlex Ultra Assay Kits</b>						
Human GM-CSF V2	K151AVAD-21	K151AVAD-22	K151AVAD-24	K151AVAG-21	K151AVAG-22	K151AVAG-24
Human IFN- $\gamma$ V2	K151AVCD-21	K151AVCD-22	K151AVCD-24	K151AVCG-21	K151AVCG-22	K151AVCG-24
Human IL-1 $\beta$ V2	K151AWCD-21	K151AWCD-22	K151AWCD-24	K151AWCG-21	K151AWCG-22	K151AWCG-24
Human IL-6 V2	K151AVED-21	K151AVED-22	K151AVED-24	K151AVEG-21	K151AVEG-22	K151AVEG-24
Human IL-12p70 V2	K151AWDD-21	K151AWDD-22	K151AWDD-24	K151AWDG-21	K151AWDG-22	K151AWDG-24
Human IL-8 V2	K151AWFD-21	K151AWFD-22	K151AWFD-24	K151AWFG-21	K151AWFG-22	K151AWFG-24
Human IL-17A V2	K151AWGD-21	K151AWGD-22	K151AWGD-24	K151AWGG-21	K151AWGG-22	K151AWGG-24
Human TNF- $\alpha$ V2	K151AVGD-21	K151AVGD-22	K151AVGD-24	K151AVGG-21	K151AVGG-22	K151AVGG-24