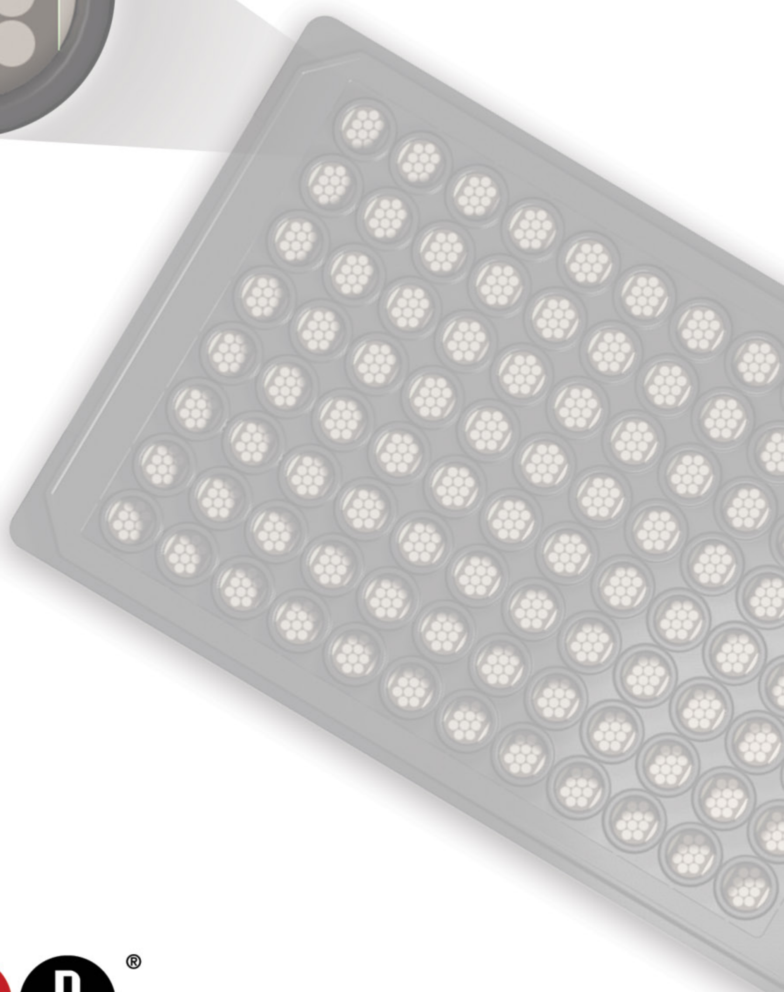
TH17 Panel 1 (human) Kits

IL-17A Gen. B, IL-21, IL-22, IL-23, IL-27, IL-31, MIP-3α



	V-PLEX®	V-PLEX Plus
TH17 Panel 1 (human)	K15085D	K15085G
Individual Assay Kits		
Human IL-17A Gen. B	K151WMD	K151WMG
Human IL-21	K151WUD	K151WUG
Human IL-22	K151WVD	K151WVG
Human IL-23	K151WWD	K151WWG
Human IL-27	K151WXD	K151WXG
Human IL-31	K151XAD	K151XAG
Human MIP-3α	K151XDD	K151XDG

MSD Cytokine Assays

TH17 Panel 1 (human) Kits

IL-17A Gen. B, IL-21, IL-22, IL-23, IL-27, IL-31, MIP-3α

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.

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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 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 TH17 Panel 1 (human) measures seven cytokines that are important in TH17 pathways. TH17 cells are a critical part of the immune system and act as a bridge between the innate and adaptive immune systems. Improper regulation of their proinflammatory activities contributes to numerous pathogenic conditions such as rheumatoid arthritis, psoriasis, type 1 diabetes, multiple sclerosis, Crohn's disease, and asthma. As a result of their association with such a wide spectrum of disease, these biomarkers are the subjects of drug discovery projects, diagnostics development, and basic research. The biomarkers constituting the panel are described below.

Human interleukin-17A (IL-17A) is a member of IL-17 family of cytokines, which also includes IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F.[1,2] IL-17 cytokines are emerging as key regulators of the initiation and maintenance of the proinflammatory immune response. These molecules have a molecular mass of 20–30 kDa and share 20–50% homology to IL-17A. In general, IL-17 induces several pro-inflammatory molecules such as IL-6, IL-8, GM-CSF, IL-1, TNF-α, and MCP-1 from different cell types including fibroblasts, endothelial cells, epithelial cells, and tissue-specific macrophages.[1,3] Thus, it makes sense that these cytokines are linked to inflammatory diseases, including rheumatoid arthritis, asthma, lupus, allograft rejection, and tumorigenicity.[1,4,5] However, differences in expression patterns and production levels appear to give specificity of action and role to each of the IL-17 family members.

Human interleukin-21 (IL-21) is a regulatory cytokine thought to play a role in the transition between innate and adaptive immunity. It is produced and secreted by a variety of CD4+ T cells, NK cells, and T follicular helper cells.[6-8] IL-21 is a regulator in the production and proliferation of B cells, T cells, and NK cells.[8] IL-21 activity is mediated by binding its receptor, IL-21R.[9] Elevated levels of T follicular helper cells in rheumatoid arthritis positively correlate with increased IL-21 mRNA expression and IL-21 concentrations in patient plasma.[10] The same phenomenon is observed in collagen-induced arthritis.[7] It has been speculated that the mechanism is indirect regulation of RANKL by IL-21, which promotes production of osteoclasts that causes joint inflammation.[7] In addition, IL-21 is significantly elevated in colitis-associated colon cancer, ulcerative colitis, and Crohn's disease, along with all inflammatory bowel diseases.[8,11] This creates a localized environment of inflammatory NK cells and other lymphocytes leading to further pathogenesis of the diseases. IL-21 is a target of novel therapeutic approaches such as the use of IL-21 blockers to prevent inflammation and/or malignancy.[11]

Human interleukin-22 (IL-22), first described as IL-10-related T cell inducible factor, is part of the IL-10 superfamily notable for its proinflammatory or anti-inflammatory actions, which are dependent on the context of its expression.[12-14] It is produced by TH17 cells and NK cells, and especially in epithelial cells like those in the skin, lung, liver, and intestine.[12,15] IL-22 binds heterodimer IL-22R that is made up of IL-22R1 and IL-10R2.[12-14] IL-22 concentration in synovial fluid and serum is higher in individuals with

rheumatoid arthritis than in osteoarthritic or normal individuals.[14] This is thought to be the result of the induction of RANKL and increased osteoclast production leading to joint inflammation.[14] In contrast, IL-22 plays a protective and anti-inflammatory role in alcohol liver injury with increased levels of IL-22 indicating reduced markers of oxidative stress, inflammation, and injury in serum.[15] Improved liver health is corroborated through histological examination.[15] In another example of anti-inflammatory properties, IL-22 is upregulated in allergic airway inflammation to attenuate the inflammatory activity of eosinophils and possibly the subsequent production of pro-inflammatory cytokines and chemokines.[13]

Human interleukin-23 (IL-23) is a heterodimeric cytokine that is structurally and functionally related to IL-12. Both share the 40 kDa (p40) subunit of IL-12, and this forms a disulfide link with a 19 kDa (p19) subunit to make the active IL-23 molecule.[16] IL-23 is produced by activated macrophages and dendritic cells in response to certain bacterial and viral pathogens and may play a key role in sustaining inflammatory responses that link innate and adaptive immunity.[17] The main role of IL-23 involves the differentiation of TH17 cells, a novel subset of CD4+ memory T cells, and their stimulation to produce the cytokine, IL-17. Elevated levels of IL-23 and IL-17 have been consistently observed in autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, and diabetes. Chronic over-expression of IL-23 may also play a role in angiogenesis and increased tumor growth.[17-19] IL-23 is a key player in sustaining cellular immunity by promoting the survival and effector cytokine production of TH1 memory cells.

Human interleukin-27 (IL-27), a heterodimeric cytokine and a relatively new member of the IL-6/IL-12 family, plays an important role in the regulation of TH1 responses.[20] IL-27 consists of two subunits, an EBV-transformed gene 3 (EBI3) subunit and a p28 cytokine subunit. IL-27 activity is mediated by binding to its receptor, IL-27R, comprised of WSX-1 and gp130.[20,21] Several immune cells co-express both subunits of the IL-27 receptor. IL-27 is expressed by antigen-presenting cells upon antigen activation. IL-27 potently induces the proliferation of naive T cells, and synergistically with IL-12 stimulates the production of IFN-γ by naive CD4+ T cells.[22] Studies indicate that IL-27 has strong antitumor and anti-metastatic abilities. IL-27 also plays a role in the induction of several antiangiogenic chemokines, such as IP-10 and MIG. These factors make IL-27 an attractive candidate for cancer immunotherapy.[23]

Human interleukin-31 (IL-31) is a four-helix bundle cytokine that belongs to the gp130/IL-6 cytokine family.[24] IL-31 is produced by TH2 cells and signals through a heterodimeric receptor composed of IL-31 receptor A (IL-31RA) and oncostatin M receptor.[25] Signaling by IL-31 activates the JAK-STAT, the RAS/ERK, and the PI3K/AKT pathways.[26] IL-31 has been associated with allergic skin conditions such as atopic dermatitis and has been shown to promote the expression of proinflammatory cytokines in irritable bowel syndrome.[27] IL-31 elevates the expression of EGF, VEGF, and MCP-1, and activates MAPK protein, which induces bronchial inflammation. Due to its active role in inflammation, IL-31 and its receptor are promising therapeutic targets for conditions including atopic dermatitis, airway hypersensitivity, and irritable bowel disease.[26,27]

Human macrophage inflammatory protein-3 alpha (MIP-3α/LARC/CCL20) is a C-C chemokine with inflammatory and homeostatic functions.[28,29] Initially identified in the liver, MIP-3α is expressed in lymphatic tissue, lung tissue, macrophages, dendritic cells, B- and T-lymphocytes, and eosinophilic granulocytes as well as in normal colon, pancreas, prostate, uterine cervix, and skin.[29] To date, MIP-3α is the only known ligand for the CCR6 receptor; it is also chemotactic for CCR6+ cells such as TH17.[28-30] MIP-3α is associated with a broad spectrum of disorders, including colorectal cancer and tumor metastasis, rheumatoid arthritis, psoriasis, obesity, and wound healing.[28,30-33] Disrupting the MIP-3α and CCR6 interaction may ultimately prove to be a viable therapeutic strategy, as CCR6 deletion resulted in a reduced atherosclerotic lesion area and reduced macrophage presence at atherosclerotic plaque sites in models of atherogenesis.[31,34]

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 TH17 Panel 1 (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-21, IL-31 and MIP-3α assays are provided on 10-spot MULTI-SPOT plates (Figure 1); the individual IL-17A Gen. B, IL-27, IL-23, and IL-22 assays are provided on Small Spot plates (Figure 2). The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that creates the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD® instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light (which is proportional to the amount of analyte present in the sample) and provides a quantitative measure of each analyte in the sample. V-PLEX assay kits have been validated according to the principles outlined in "Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement" by J. W. Lee, et al.[39]

1. IL-17A Gen. B
2. *BSA coated*
3. *BSA coated*
4. IL-21
5. IL-31
6. IL-27
7. IL-23
8. IL-22
9. *BSA coated*
10. MIP-3α

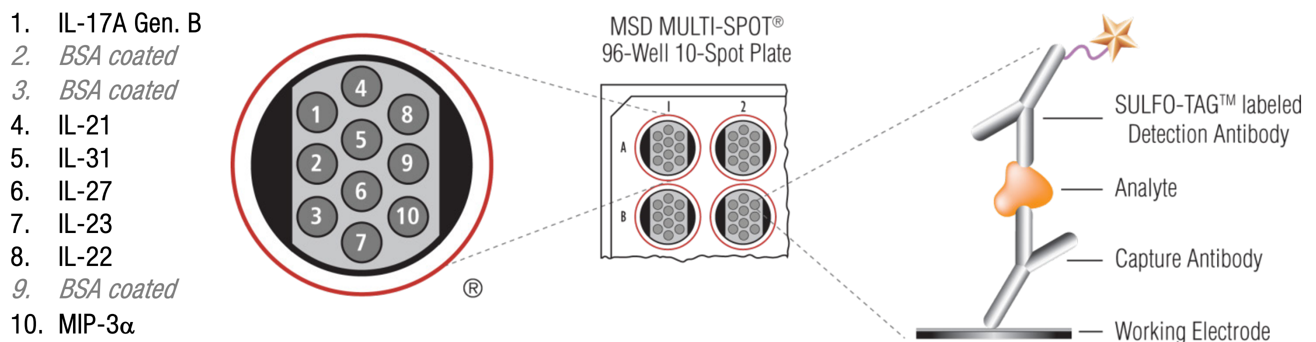

Figure 1. Multiplex plate spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

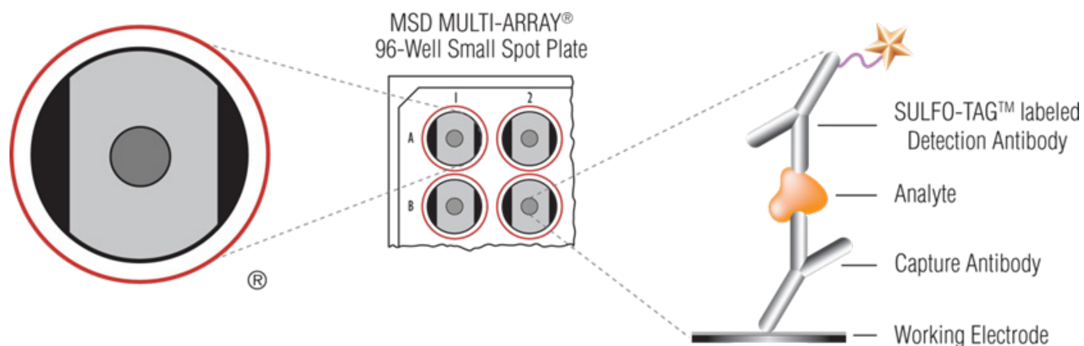

Figure 2. Small Spot plate diagram showing placement of analyte capture antibodies.

Kit Components

TH17 Panel 1 (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 No.	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	
TH17 Panel 1 (human) Calibrator Blend	2–8 °C	C0085-2	1 vial	1 vial	5 vials	25 vials	Seven 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 No.	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	
TH17 Panel 1 (human) Control 1*	2–8 °C	C4085-1	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.
TH17 Panel 1 (human) Control 2*	2–8 °C	C4085-1	1 vial	1 vial	5 vials	25 vials	
TH17 Panel 1 (human) Control 3*	2–8 °C	C4085-1	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 TH17 Panel 1 (human) Control Pack (catalog no. C4085-1)

Kit-Specific Components

Table 3. Components that are supplied with specific kits

Plates	Storage	Part No.	Size	Quantity Supplied 1-Plate Kit	5-Plate Kit	25-Plate Kit	Description
TH17 Panel 1 (human) SECTOR™ Plate	2–8 °C	N05085A-1	10-spot	1	5	25	96-well plate, foil sealed, with desiccant.
Human IL-17A Gen. B SECTOR Plate	2–8 °C	L451WMA-1	Small Spot	1	5	25	
Human IL-22 SECTOR Plate	2–8 °C	L451WVA-1	Small Spot	1	5	25	
Human IL-23 SECTOR Plate	2–8 °C	L451WWA-1	Small Spot	1	5	25	
Human IL-27 SECTOR Plate	2–8 °C	L451WXA-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 No.	Size	Quantity Supplied 1-Plate Kit	5-Plate Kit	25-Plate Kit	Description
Anti-hu IL-17A Antibody (50X)	2–8 °C	D21WM-2	75 µL	1			SULFO-TAG conjugated antibody.
		D21WM-3	375 µL		1	5	
Anti-hu IL-21 Antibody (50X)	2–8 °C	D21WU-2	75 µL	1			SULFO-TAG conjugated antibody.
		D21WU-3	375 µL		1	5	
Anti-hu IL-22 Antibody (50X)	2–8 °C	D21WV-2	75 µL	1			SULFO-TAG conjugated antibody.
		D21WV-3	375 µL		1	5	
Anti-hu IL-23 Antibody (50X)	2–8 °C	D21WW-2	75 µL	1			SULFO-TAG conjugated antibody.
		D21WW-3	375 µL		1	5	
Anti-hu IL-27 Antibody (50X)	2–8 °C	D21WX-2	75 µL	1			SULFO-TAG conjugated antibody.
		D21WX-3	375 µL		1	5	
Anti-hu IL-31 Antibody (50X)	2–8 °C	D21XA-2	75 µL	1			SULFO-TAG conjugated antibody.
		D21XA-3	375 µL		1	5	
Anti-hu MIP-3α Antibody (50X)	2–8 °C	D21XD-2	75 µL	1			SULFO-TAG conjugated antibody.
		D21XD-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 500–1000 rpm
- ❑ Phosphate-buffered saline (PBS) plus 0.05% Tween-20 for plate washing or MSD Wash Buffer catalog no. 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

- ❑ TH17 Panel 1 (human) Control Pack, available for separate purchase from MSD, catalog no. C4085-1 (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 applicable safety data sheet(s) (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

Best Practices

- Do not mix or substitute reagents from different sources or different kit lots. Lot information is provided in the lot-specific COA.
- Assay incubation steps should be performed between 20–26 °C to achieve the most consistent signals between runs.
- Bring frozen diluent to room temperature in a 24 °C water bath. Thaw other reagents on wet ice and use as directed without delay.
- Prepare calibrators, samples, and controls in polypropylene microcentrifuge tubes; use a fresh pipette tip for each dilution; vortex after each dilution before proceeding.
- Samples and standards should be run in duplicates.
- Avoid prolonged exposure of detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light except for direct sunlight.
- Avoid bubbles in wells at all pipetting steps. Bubbles may lead to variable results; bubbles introduced when adding Read Buffer T may interfere with signal detection.
- Do not touch the pipette tip on the bottom of the wells when pipetting into the MSD plate.
- Use reverse pipetting when necessary to avoid introduction of bubbles. For empty wells, pipette to the bottom corner.
- Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm. Binding reactions may reach equilibrium sooner if you use shaking at the middle of this range (~700 rpm) or above.
- When using an automated plate washer, rotate the plate 180 degrees between wash steps to improve assay precision.
- Gently tap the plate on a paper towel to remove residual fluid after washing.
- Read buffer should be at room temperature when added to the plate.
- Keep time intervals consistent between adding read buffer and reading the plate to improve inter-plate precision. Unless otherwise directed, read plate as soon as practical after adding read buffer.
- No shaking is necessary after adding read buffer.
- If an incubation step needs to be extended, avoid letting the plate dry out by keeping sample or detection antibody solution in the plate.
- Remove the plate seals prior to reading the plate.
- If assay results are above the top of the calibration curve, dilute the samples and repeat the assay.
- When running a partial plate, seal the unused sectors to avoid contaminating unused wells. Remove all seals before reading. The uncoated wells of a partially used plate may be sealed and stored up to 30 days at 2–8 °C in the original foil pouch with desiccant. You may adjust volumes proportionally when preparing reagents.

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.

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:

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.

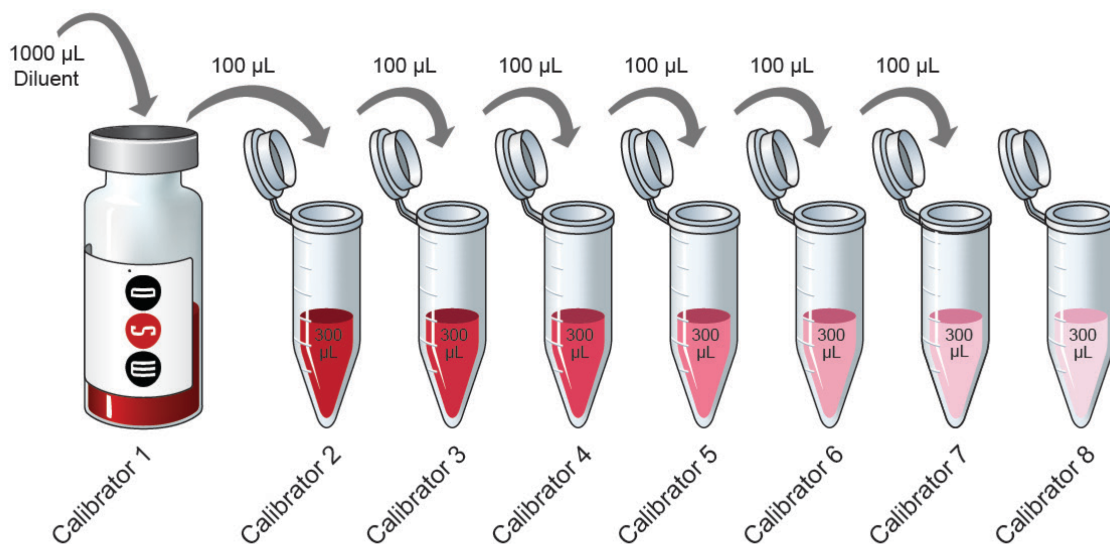

***Figure 3**. Dilution schema for preparation of Calibrator Standards*

Sample Collection and Handling

Below are general guidelines for human sample collection, storage, and handling. If possible, use published guidelines.[35-38] 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 °C 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 50 µL of sample to 150 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. 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 TH17 Panel 1 (human) Control Pack, catalog no. C4085-1. (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.

10-plex TH17 Panel 1 (human) kit

For one plate, combine the following detection antibodies and add to 2,580 µL of Diluent 3:

- ❑ 60 µL of SULFO-TAG Anti-hu IL-17A Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu IL-21 Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu IL-31 Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu IL-27 Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu IL-23 Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu IL-22 Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu MIP-3α 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 °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 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).

Validation

MSD's V-PLEX products are validated following fit-for-purpose principles[39] 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 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, blood, 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 TH17 Panel 1 (human) were collected over two months of testing by five operators (23 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.

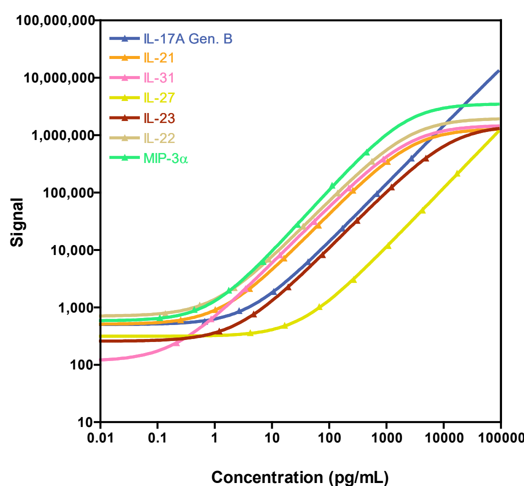

Figure 4. Typical calibration curves for the TH17 Panel 1 (human) assay

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% (<25% for IL-21) and the recovery of each analyte is within 80% to 120% (75% to 125% for IL-21) of the known value.

The LLOQ is the lowest concentration at which the CV of the calculated concentration is <20% (<25% for IL-21) and the recovery of each analyte is within 80% to 120% (75% to 125% for IL-21) of the known value.

The quantitative range of the assay lies between the LLOQ and ULOQ.

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

Table 5. LLOD, LLOQ, and ULOQ for each analyte in the TH17 Panel 1 (human) Kit

	Median LLOD (pg/mL)	LLOD Range (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
IL-17A Gen. B	0.413	0.148–2.15	5.86	1,950
IL-21	0.193	0.059–1.20	6.12	650
IL-22	0.270	0.040–1.99	2.78	325
IL-23	0.274	0.041–1.99	4.60	3,250
IL-27	4.20	1.86–29.0	38.7	13,000
IL-31	0.446	0.218–1.08	4.22	650
MIP-3α	0.050	0.020–0.270	0.750	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 five operators using a minimum of three replicates on 60 runs over 10 days. 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 across 60 runs (four kit lots).

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 4 kit lots (total of 60 runs).

Table 6. Intra-run and Inter-run %CVs for each analyte in the TH17 Panel 1 (human) Kit

	Control	Average Conc. (pg/mL)	Average Intra-run %CV	Inter-run %CV	Inter-lot %CV
IL-17A Gen. B	Control 1	2,810	4.1	6.6	5.8
	Control 2	407	3.9	6.7	7.1
	Control 3	59	4.9	10.4	13.7
IL-21	Control 1	824	2.9	7.8	7.4
	Control 2	150	4.1	8.8	7.8
	Control 3	24	4.5	13.0	11.4
IL-22	Control 1	530	3.5	5.6	6.3
	Control 2	92	3.9	6.6	7.9
	Control 3	16	4.2	12.3	12.2
IL-23	Control 1	3,967	3.6	7.7	7.4
	Control 2	453	4.0	8.8	9.3
	Control 3	51	6.1	10.7	13.7
IL-27	Control 1	15,231	2.8	8.4	12.2
	Control 2	3,181	2.9	7.6	13.3
	Control 3	1,044	3.7	9.6	16.3
IL-31	Control 1	993	3.2	7.1	8.3
	Control 2	150	2.9	7.0	8.4
	Control 3	24	3.3	8.4	11.4
MIP-3α	Control 1	501	2.9	6.6	12.3
	Control 2	196	3.1	7.8	14.5
	Control 3	64	5.0	10.8	17.5

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 2-fold, 4-fold, 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 Gen. B		IL-21		IL-22		IL-23	
Sample Type	Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Serum (N=10)	2	91	85–95	101	94–112	88	85–90	103	90–116
	4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	8	104	96–111	92	82–105	104	97–111	105	96–118
	16	105	97–113	94	83–107	107	98–116	111	91–140
	32	104	95–112	94	74–110	103	95–107	133	99–190
EDTA Plasma (N=10)	2	107	92–122	113	84–143	96	88–106	102	88–121
	4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	8	96	90–104	99	87–118	101	94–107	91	51–113
	16	96	84–111	114	85–148	103	86–117	90	66–106
	32	97	79–118	127	81–183	103	74–116	103	80–139
Citrate Plasma (N=10)	2	100	79–109	104	74–124	92	84–101	92	75–105
	4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	8	99	87–116	103	86–123	104	89–116	106	98–119
	16	106	83–143	111	85–155	109	94–122	107	79–116
	32	111	80–166	120	76–182	109	96–136	121	83–151
Urine (N=10)	2	98	93–103	91	73–105	97	90–109	90	68–112
	4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	8	96	91–100	100	95–106	98	94–109	106	96–120
	16	100	94–109	102	95–114	102	90–124	103	93–114
	32	98	93–104	102	95–117	110	96–131	107	97–117
Heparin Plasma (N=10)	2	98	93–107	39	37–41	94	88–103	100	81–128
	4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	8	101	94–109	171	151–190	100	94–114	127	99–164
	16	99	93–106	232	200–258	100	82–120	141	96–190
	32	102	97–109	267	227–308	106	98–116	171	102–226
Cell Culture Supernatant (N=6)	2	100	95–110	97	86–108	93	57–102	99	78–112
	4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	8	94	90–100	94	84–103	94	70–103	96	62–118
	16	98	93–103	97	86–118	93	61–113	89	53–113
	32	97	89–118	98	85–109	102	93–110	96	89–116

Table 7 continued.

		IL-27		IL-31		MIP-3α	
Sample Type	Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Serum (N=10)	2	93	88–102	87	76–94	95	84–101
	4	N/A	N/A	N/A	N/A	N/A	N/A
	8	104	94–112	100	95–107	104	94–114
	16	104	87–118	104	99–118	107	95–128
	32	115	101–132	98	88–121	125	108–152
EDTA Plasma (N=10)	2	99	89–106	98	80–114	97	38–115
	4	N/A	N/A	N/A	N/A	N/A	N/A
	8	98	93–110	89	78–111	99	90–137
	16	104	91–124	94	76–121	93	68–149
	32	110	84–132	96	71–123	100	70–163
Citrate Plasma (N=10)	2	97	88–106	95	83–102	99	89–111
	4	N/A	N/A	N/A	N/A	N/A	N/A
	8	103	92–114	92	83–109	98	93–104
	16	109	89–148	97	83–123	93	82–104
	32	119	86–200	95	76–142	97	86–113
Urine (N=10)	2	91	75–109	92	82–99	89	60–102
	4	N/A	N/A	N/A	N/A	N/A	N/A
	8	103	97–111	100	95–106	101	95–112
	16	105	94–121	104	97–111	99	92–114
	32	109	101–119	101	91–111	101	91–121
Heparin Plasma (N=10)	2	70	58–87	92	75–102	91	71–103
	4	N/A	N/A	N/A	N/A	N/A	N/A
	8	113	100–131	104	97–116	104	95–118
	16	122	99–185	108	99–132	102	90–123
	32	132	102–188	107	95–139	102	87–136
Cell Culture Supernatant (N=6)	2	88	75–96	95	74–103	92	72–102
	4	N/A	N/A	N/A	N/A	N/A	N/A
	8	101	69–114	96	80–101	98	61–110
	16	103	87–118	99	81–105	101	63–123
	32	116	100–127	102	94–114	117	100–132

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 %CV and % 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=10)			EDTA Plasma (N=10)			Cell Culture Media (N=6)		
	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range
IL-17A Gen. B	86	6.0	78–92	91	10.7	79–111	97	3.7	94–103
IL-21	101	10.5	85–115	38	53.6	15–82	105	3.7	100–110
IL-22	89	9.1	76–104	107	6.4	94–119	95	4.0	88–98
IL-23	44	38.1	19–72	90	7.9	77–101	96	3.8	92–102
IL-27	74	28.2	47–120	90	9.4	75–102	95	4.5	90–99
IL-31	84	9.3	70–93	91	15.2	64–112	101	3.3	97–105
MIP-3α	71	18.8	43–88	76	22.6	34–92	85	3.5	82–89

	Citrate Plasma (N=10)			Heparin Plasma (N=10)			Urine (N=10)		
	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range
IL-17A Gen. B	76	7.7	66–87	98	13.8	77–117	111	4.5	102–118
IL-21	35	64.1	12–60	41	18.8	24–50	107	9.2	84–119
IL-22	87	6.5	82–101	99	13.1	83–119	102	8.2	91–111
IL-23	57	26.5	32–75	46	41.2	25–80	98	11	82–116
IL-27	75	14.1	55–90	89	28.3	48–147	110	10.1	94–130
IL-31	84	9	67–90	100	14	77–118	102	7.8	89–113
MIP-3α	74	14.4	52–93	107	20	60–142	117	16.9	85–139

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, 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-A, 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 ≤-70 °C. Partially used MSD plates may be sealed and stored up to 30 days at 2–8 °C 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 No.	NIBSC (IU/mL): MSD (pg/mL)
IL-17A	01/420	0.015

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 TH17 Panel 1 (human) Kit

Sample Type	Statistic	IL-17A Gen. B	IL-21	IL-31	IL-27	IL-23	IL-22	MIP-3α
Serum (N=30)	Median (pg/mL)	2.24	0.67	0.14	1,509	ND	1.20	4
	Range (pg/mL)	0.90–51.4	0.40–5.70	0.10–0.40	470–3,713	NA	0.50–519	1.50–15.4
	% of Samples Detected	60	57	17	100	0	30	100
EDTA Plasma (N=30)	Median (pg/mL)	12.1	36.8	2.05	1140	2.3	2.65	6.25
	Range (pg/mL)	3.60–101	20.8–86.9	0.60–34.8	297–4,013	1.50–3.30	0.90–18.5	3.00–25.5
	% of Samples Detected	100	100	100	100	10	93	100
Citrate (N=30)	Median (pg/mL)	24.5	48.4	1.35	1936	1.79	2.63	8.31
	Range (pg/mL)	2.01–53.4	24.1–142	0.50–12.8	853–3,395	1.50–7.60	0.80–11.5	4.20–35.9
	% of Samples Detected	100	100	100	100	30	100	100
Urine (N=10)	Median (pg/mL)	2.5	ND	0.1	ND	1.85	ND	2.75
	Range (pg/mL)	1.20–4.50	NA	0.10–0.20	NA	1.70–2.00	NA	1.10–4.40
	% of Samples Detected	100	0	70	0	20	0	20
Heparin (N=30)	Median (pg/mL)	1.21	6	0.14	1543	ND	0.56	6.34
	Range (pg/mL)	0.40–1.80	0.70–31.2	0.10–0.20	749–4,672	NA	0.10–7.10	2.60–91.6
	% of Samples Detected	47	80	10	100	0	53	100

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

Stimulated Samples

Cell models were treated with various stimulants for a given period of time. These cell models were tested for the presence of TH17 Panel 1 analytes. All analytes were expressed at levels higher than the detection limits in at least one of the tested samples. The dilution-adjusted concentrations (pg/mL) for each stimulation model are displayed below. All assays showed a significant difference in analyte level with various stimulation conditions.

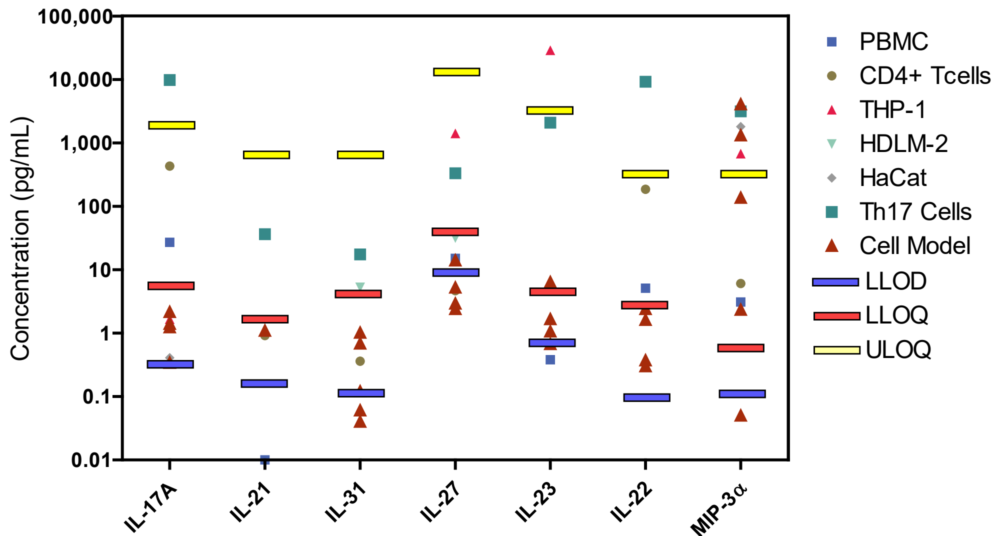

Figure 5. *Effect of cell stimulation on cytokine production as measured in the TH17 Panel 1 (human) Kit*

Samples from individuals with psoriasis, fibromyalgia, osteoarthritis, asthma, COPD, IgE allergen and anti-TPO were tested for the TH17 Panel 1 analyte levels. The dilution-adjusted concentrations (pg/mL) for each sample are displayed below.

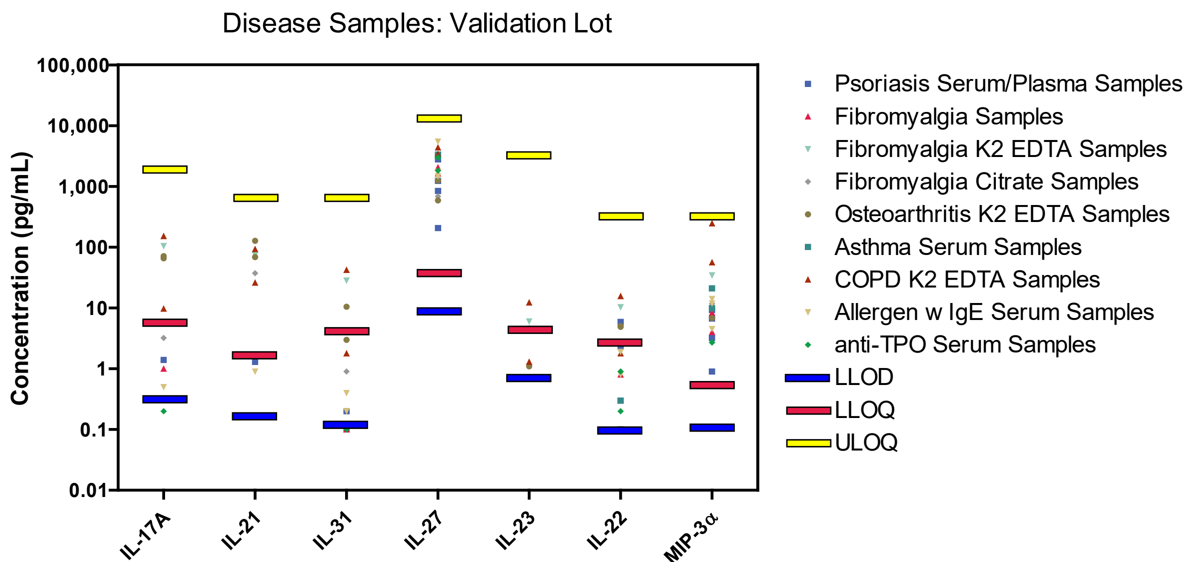

Figure 6. *Expression level of cytokine, measured using the TH17 Panel 1 (human) Kit, in samples from individuals with various diseases*

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 Gen. B	*E. coli*
IL-21	*E. coli*
IL-22	*E. coli*
IL-23	Insect cell line
IL-27	Mouse cell line
IL-31	*E. coli*
MIP-3α	*E. coli*

Antibodies

Table 12. Antibody source species

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
IL-17A Gen. B	Mouse Monoclonal	Mouse Monoclonal	B
IL-21	Mouse Monoclonal	Mouse Monoclonal	A
IL-22	Rat Monoclonal	Rat Monoclonal	A
IL-23	Mouse Monoclonal	Mouse Monoclonal	A
IL-27	Mouse Monoclonal	Mouse Monoclonal	A
IL-31	Mouse Monoclonal	Mouse Monoclonal	A
MIP-3α	Mouse Monoclonal	Goat Polyclonal	A

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Appendix A

Calibration curves below illustrate the relative sensitivity for each assay under **Alternate Protocols**: 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).

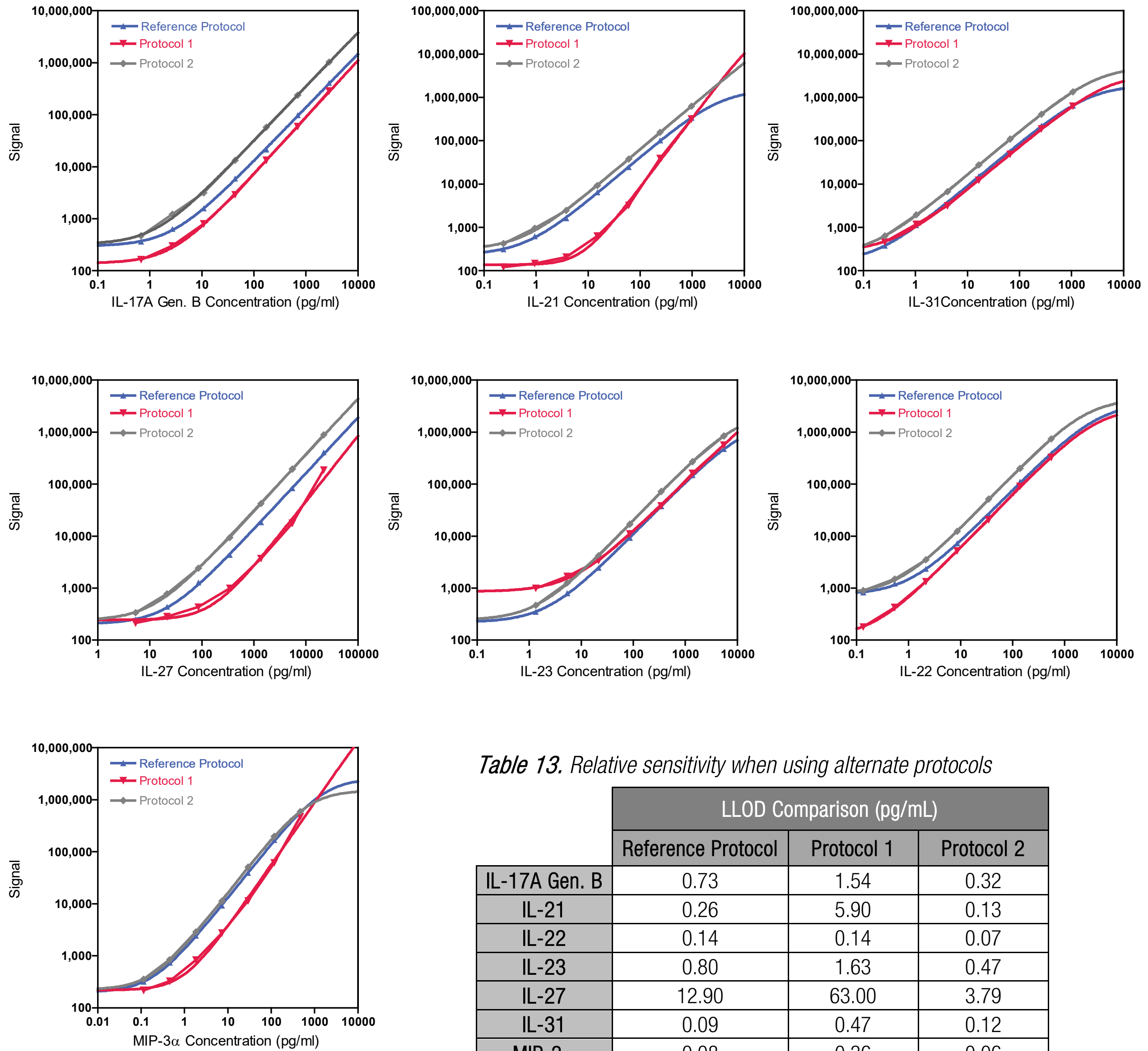

Table 13. Relative sensitivity when using alternate protocols

	LLOD Comparison (pg/mL)		
	Reference Protocol	Protocol 1	Protocol 2
IL-17A Gen. B	0.73	1.54	0.32
IL-21	0.26	5.90	0.13
IL-22	0.14	0.14	0.07
IL-23	0.80	1.63	0.47
IL-27	12.90	63.00	3.79
IL-31	0.09	0.47	0.12
MIP-3α	0.08	0.36	0.06

Appendix B

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

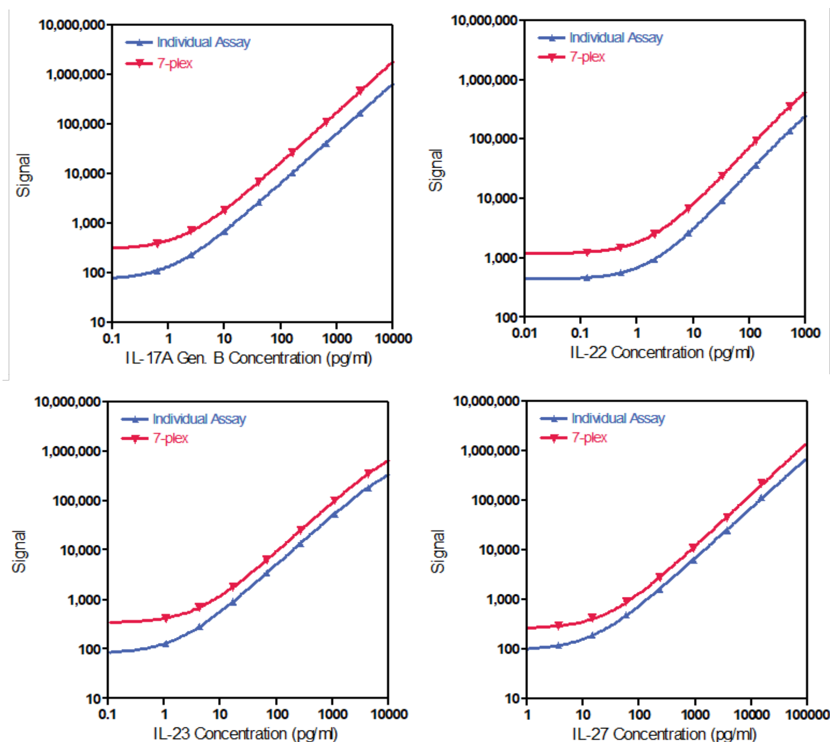

Table 14. Assay performance for individual and 7-plex assays

In general, assays in the single-spot format yielded a lower overall signal compared to the 7-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-21, IL-31 and MIP-3α are not included since the individual assays are run on multiplex plates.

	LLOD (pg/mL)	
Assay	Individual	7-plex
IL-17A Gen. B	0.43	0.51
IL-22	0.10	0.39
IL-23	0.56	0.94
IL-27	3.54	7.03

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 (red curve) vs. running each assay using a single, assay-specific detection antibody (blue curve).

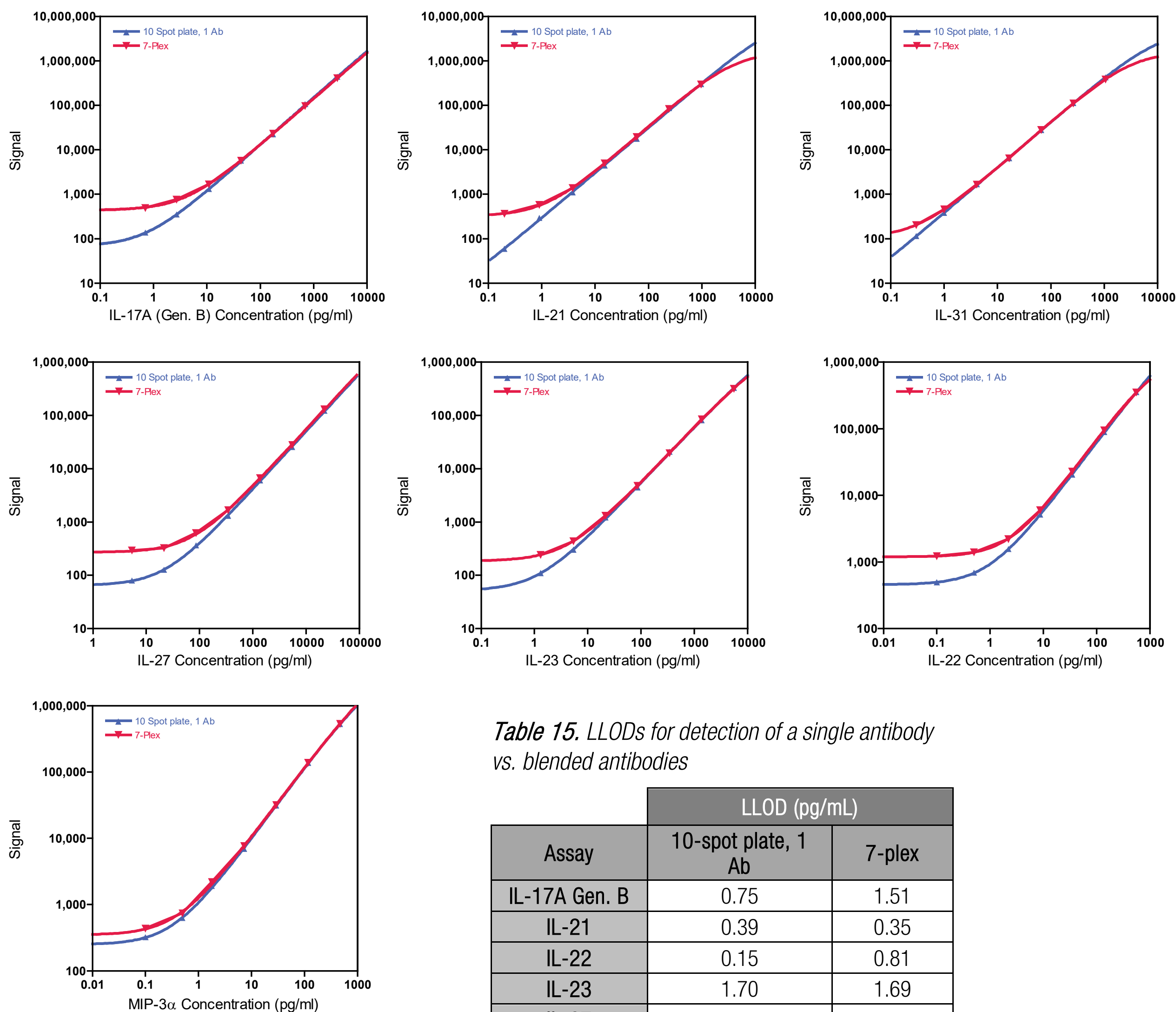

Table 15. LLODs for detection of a single antibody vs. blended antibodies

	LLOD (pg/mL)	
Assay	10-spot plate, 1 Ab	7-plex
IL-17A Gen. B	0.75	1.51
IL-21	0.39	0.35
IL-22	0.15	0.81
IL-23	1.70	1.69
IL-27	16.32	14.32
IL-31	0.21	0.22
MIP-3α	1.34	0.15

Summary Protocol

TH17 Panel 1 (human) Kits

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the TH17 Panel 1 (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 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						
TH17 Panel 1 (human)	K15085D-1	K15085D-2	K15085D-4	K15085G-1	K15085G-2	K15085G-4
Individual Assay Kits						
Human IL-17A Gen. B	K151WMD-1	K151WMD-2	K151WMD-4	K151WMG-1	K151WMG-2	K151WMG-4
Human IL-21	K151WUD-1	K151WUD-2	K151WUD-4	K151WUG-1	K151WUG-2	K151WUG-4
Human IL-22	K151WVD-1	K151WVD-2	K151WVD-4	K151WVG-1	K151WVG-2	K151WVG-4
Human IL-23	K151WWD-1	K151WWD-2	K151WWD-4	K151WWG-1	K151WWG-2	K151WWG-4
Human IL-27	K151WXD-1	K151WXD-2	K151WXD-4	K151WXG-1	K151WXG-2	K151WXG-4
Human IL-31	K151XAD-1	K151XAD-2	K151XAD-4	K151XAG-1	K151XAG-2	K151XAG-4
Human MIP-3α	K151XDD-1	K151XDD-2	K151XDD-4	K151XDG-1	K151XDG-2	K151XDG-4

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

Plate Diagram

12 11 10 9 8 7 6 5 4 3 2 1

A B C D E F G H