Proinflammatory Panel 1 (human) Kit

IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-17A, TNF-α

S-PLEX®
TrueSensitivity®
with TURBO-BOOST® & TURBO-TAG®
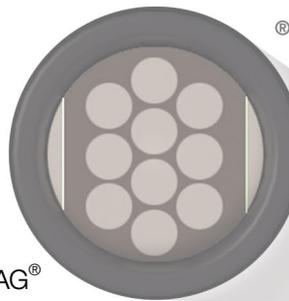
Proinflammatory Panel 1 (human)

Catalog No.

K15396S
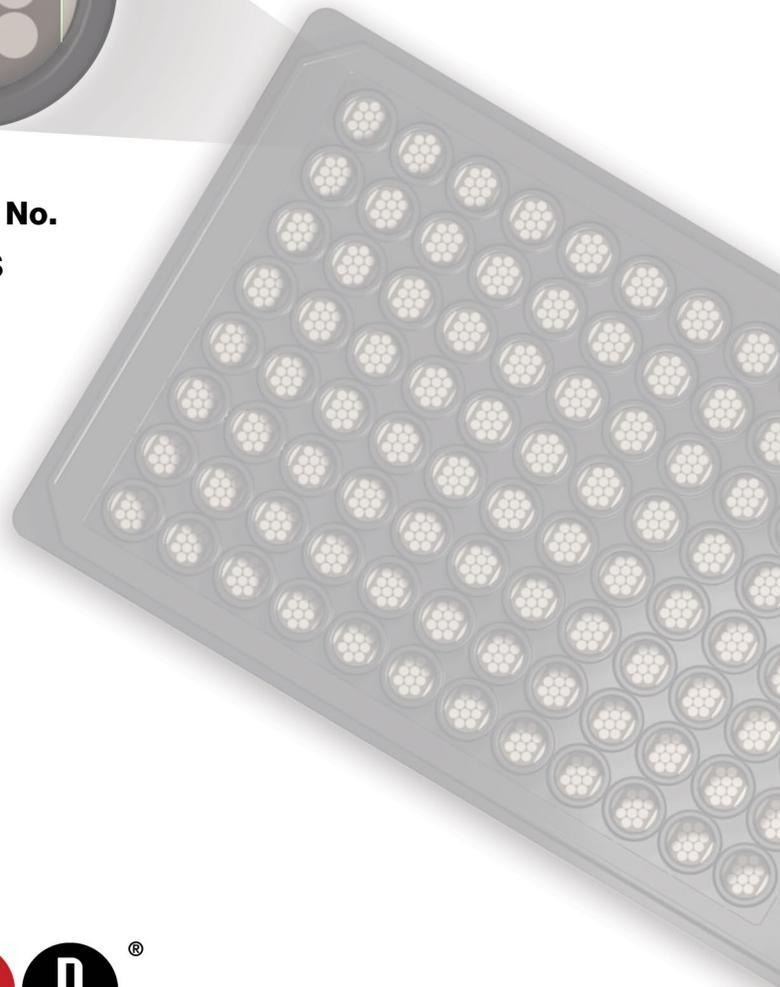
MSD S-PLEX Platform

S-PLEX® Proinflammatory Panel 1 (human) Kit

IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-17A, TNF-α

For use with human serum, plasma, cerebral spinal fluid (CSF), urine, 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 instruments

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®

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Introduction

The S-PLEX Proinflammatory Panel 1 (human) kit employs S-PLEX technology in a multiplex assay format. The kit measures nine cytokines that are important in the inflammatory response, immune system regulation, and many other biological processes. These are: Interferon-gamma (**IFN-γ**), Interleukin-1 beta (**IL-1β**), Interleukin-2 (**IL-2**), Interleukin-4 (**IL-4**), Interleukin-6 (**IL-6**), Interleukin-10 (**IL-10**), Interleukin-12p70 (**IL-12p70**), Interleukin-17A (**IL-17A**), and Tumor necrosis factor-alpha (**TNF-α**).

S-PLEX is MSD's ultrasensitive assay platform. It can dramatically improve immunoassays' sensitivities, reducing the lower limit of detection (LLOD) by 10- to 1000-fold over other assay methods. Results vary from assay to assay, but detection limits in the low femtogram/mL range are common. These low detection limits reduce the required sample volume and reduce the use of critical reagents.

S-PLEX uses electrochemiluminescence (ECL) technology, retaining its well-known advantages and superior analytical performance. The improved sensitivity of S-PLEX is due, in part, to the new TURBO-TAG® and TURBO-BOOST® reagents. When TURBO-TAG is combined with an antibody labeled with TURBO-BOOST, more signal is generated when compared to other ECL formats that use SULFO-TAG™ as the detection label. The S-PLEX platform uses the same robust MSD® instruments as other MSD assays. The protocol for S-PLEX is also straightforward, similar to other MSD assay methods. It is comprised of three simple steps: (1) Assemble the immunoassay, (2) Enhance with a TURBO-TAG label, and (3) Read on an MSD instrument. S-PLEX assays are available in both single-assay and multiplex formats.

Principle of the Assay

The assays in the S-PLEX Proinflammatory Panel 1 (human) are sandwich immunoassays. Biotinylated capture antibodies are coupled to linkers, which self-assemble onto unique spots on the S-PLEX Multiplex 96-Well SECTOR Plate. Analytes in the sample bind to the capture reagents; detection antibodies conjugated with TURBO-BOOST label bind to the analytes to complete the sandwich immunoassay (Figure 1). Once the sandwich immunoassay is complete, the TURBO-TAG label detect solution is added to enhance TURBO-BOOST labeled detection antibody. The user adds an MSD read buffer that creates the 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.

1. IL-6
2. IL-10
3. IL-12p70
4. IL-4
5. TNF-α
6. IL-2
7. –
8. IL-1β
9. IFN-γ
10. IL-17A
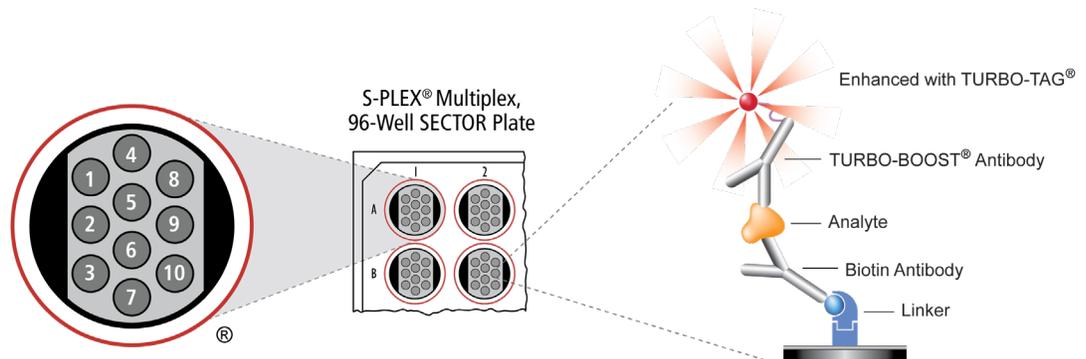
Figure 1. Spot diagram of multiplex plate showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Kit Components

S-PLEX Proinflammatory Panel 1 (human) assays are available as multiplex and custom kits with subsets of assays selected from the full panel. Kits have kit lot-specific (Table 1) and non-kit lot-specific regents (Table 2, Table 3). 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 1. *Reagents and components that are supplied with the S-PLEX Proinflammatory Panel 1 (human) kit*

Reagent	Cap color	Storage	Catalog No.	Size	Quantity Supplied			Description
					1 Plate	5 Plates	25 Plates	
S-PLEX Proinflammatory Panel 1 (human) Coating Solution	⚪	≤−70 °C	C2396-2	1.7 mL	1 vial	5 vial	25 vials	Blended biotinylated capture antibody bound with spot-specific linkers
TURBO-BOOST Human IFN-γ Antibody	🟢	2–8 °C	D21AEB-2	45 µL	1 vial	–	–	TURBO-BOOST conjugated detection antibody
			D21AEB-3	225 µL	–	1 vial	5 vials	
TURBO-BOOST Human IL-1β Antibody	🟢	2–8 °C	D21AEA-2	45 µL	1 vial	–	–	TURBO-BOOST conjugated detection antibody
			D21AEA-3	225 µL	–	1 vial	5 vials	
TURBO-BOOST Human IL-2 Antibody	🟢	2–8 °C	D21ADY-2	45 µL	1 vial	–	–	TURBO-BOOST conjugated detection antibody
			D21ADY-3	225 µL	–	1 vial	5 vials	
TURBO-BOOST Human IL-4 Antibody	🟢	2–8 °C	D21ADX-2	45 µL	1 vial	–	–	TURBO-BOOST conjugated detection antibody
			D21ADX-3	225 µL	–	1 vial	5 vials	
TURBO-BOOST Human IL-6 Antibody	🟢	2–8 °C	D21ADU-2	45 µL	1 vial	–	–	TURBO-BOOST conjugated detection antibody
			D21ADU-3	225 µL	–	1 vial	5 vials	
TURBO-BOOST Human IL-10 Antibody	🟢	2–8 °C	D21ADV-2	45 µL	1 vial	–	–	TURBO-BOOST conjugated detection antibody
			D21ADV-3	225 µL	–	1 vial	5 vials	
TURBO-BOOST Human IL-12p70 Antibody	🟢	2–8 °C	D21ADW-2	45 µL	1 vial	–	–	TURBO-BOOST conjugated detection antibody
			D21ADW-3	225 µL	–	1 vial	5 vials	
TURBO-BOOST Human IL-17A Antibody	🟢	2–8 °C	D21AEC-2	45 µL	1 vial	–	–	TURBO-BOOST conjugated detection antibody
			D21AEC-3	225 µL	–	1 vial	5 vials	
TURBO-BOOST Human TNF-α Antibody	🟢	2–8 °C	D21ADZ-2	45 µL	1 vial	–	–	TURBO-BOOST conjugated detection antibody
			D21ADZ-3	225 µL	–	1 vial	5 vials	
S-PLEX Proinflammatory Panel 1 (human) Calibrator Blend	–	2–8 °C	C0396-2	1 vial	1 vial	5 vials	25 vials	Lyophilized assay calibrator
Blocker S1 (100X)	⚫	≤−10 °C	R93AG-1	500 µL	1 vial	1 vial	5 vials	Added to assay diluent, reduces nonspecific signals.
Blocker S2 (100X)	🔴	≤−10 °C	R93AH-1	500 µL	1 vial	1 vial	5 vials	Added to assay diluent, reduces nonspecific signals.
S-PLEX Enhance E1 (4X)	🟡	≤−10 °C	R82AA-1	1.7 mL	1 vial	5 vials	25 vials	Reagent 1 of 3 for Enhance Step
S-PLEX Enhance E2 (4X)	🟠	≤−10 °C	R82AB-1	1.7 mL	1 vial	5 vials	25 vials	Reagent 2 of 3 for Enhance Step
S-PLEX Enhance E3 (200X)	🔴	≤−70 °C	R82AC-1	50 µL	1 vial	5 vials	25 vials	Reagent 3 of 3 for Enhance Step
S-PLEX Detect D1 (4X)	🟣	≤−70 °C	D20K0-2	1.7 mL	1 vial	5 vials	25 vials	Reagent 1 of 2 for Detection Step (contains TURBO-TAG label)
S-PLEX Detect D2 (200X)	🔵	≤−70 °C	D20J0-2	50 µL	1 vial	5 vials	25 vials	Reagent 2 of 2 for Detection Step

Reagent	Cap color	Storage	Catalog No.	Size	Quantity Supplied 1 Plate	Quantity Supplied 5 Plates	Quantity Supplied 25 Plates	Description
Diluent 58	–	≤–10 °C	R50CA-1	10 mL	1 bottle	–	–	Assay diluent for samples and calibrator
			R50CA-1	50 mL	–	1 bottle	5 bottles	

Dash (–) = not applicable

Non-Kit Lot-Specific Reagents and Components

Table 2. Reagents and components that are supplied with the S-PLEX Proinflammatory Panel 1 (human) kit

Reagent	Storage	Catalog No.	Size	Quantity Supplied 1 Plate	Quantity Supplied 5 Plates	Quantity Supplied 25 Plates	Description
Diluent 100	2–8 °C	R50AA-4	50 mL	1 bottle	1 bottle	5 bottles	Coating buffer for capture antibody and S-PLEX Coating Reagent C1
Diluent 59	2–8 °C	R50CB-2	8 mL	1 bottle	–	–	Antibody diluent for diluting the TURBO-BOOST Antibody
		R50CB-4	40 mL	–	1 bottle	5 bottles	
MSD GOLD™ Read Buffer B	RT	R60AM-1	18 mL	1 bottle	–	–	Buffer to catalyze the electrochemiluminescence reaction
		R60AM-2	90 mL	–	1 bottle	5 bottles	

Dash (–) = not applicable

Table 3. Plates that are supplied with the S-PLEX Proinflammatory Panel 1 (human) kit and their instrument compatibility

Reagent	Storage	Catalog No.	Quantity Supplied 1 Plate	Quantity Supplied 5 Plates	Quantity Supplied 25 Plates	Instrument Compatibility	Description
S-PLEX Multiplex 96-Well SECTOR Plate	2–8 °C	N05396A-1	1 plate	5 plates	25 plates	MESO SECTOR S 600 MESO SECTOR S 600MM MESO QuickPlex SQ 120 MESO QuickPlex SQ 120MM	Plates for coating with capture antibodies

Dash (–) = not applicable

Additional Materials and Equipment

Materials

- Adhesive plate seals
- Micropipettes with filtered tips
- Tubes (polypropylene microcentrifuge tubes, conical tubes, library tubes)
- Serological pipettes and pipette controller
- Reagent reservoir
- Plastic bottles
- Wet ice and ice bucket
- Deionized water
- Molecular biology grade water
- MSD Wash Buffer (catalog no. R61AA-1) used at 1X
- Phosphate-buffered saline (PBS) plus 0.05% Tween-20 (PBS-T)

Equipment

- Microtiter plate shaker capable of shaking at 500–1,000 rpm
- Microtiter plate shaker capable of shaking at 500–1,000 rpm and maintaining a controlled temperature of 27 °C (e.g., Kisker heated plate shaker)
- Plate-washing equipment (automated plate washer or multi-channel pipette)
- Vortex mixer
- Water bath
- Microcentrifuge

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® website.

⚠️ CAUTION: IL-1β is detected at a high level in saliva. Take precautionary measures by using appropriate PPE such as gloves, face masks, lab coats, and hair covering to prevent contamination of kit reagents while running this assay.

Best Practices

- Mixing or substituting reagents from different sources or different kit lots is not recommended. Lot information is provided in the lot-specific COA.

- Bring frozen diluents, E1, E2, and D1 reagents to room temperature in a 22–25 °C water bath before use. If a controlled water bath is not available, thaw at room temperature. Ensure that diluents, E1, E2, and D1 reagents are fully thawed and equilibrated to room temperature before use. Mix well after thawing and before use.

- Thaw frozen vials of E3 and D2 reagents on ice until needed. Ensure that E3 and D2 reagents are fully thawed before use. Mix well after thawing and before use.

- To avoid cross-contamination between vials, open vials for each protocol step one at a time (vial caps are color-coded), use filtered pipette tips, and use a fresh pipette tip for each reagent addition.

- Prepare Calibrators and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by vortexing after each dilution.

- 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.

- 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.

- 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 a new adhesive plate seal for each incubation step.

- When washing S-PLEX assays, the best results are obtained by using a low-dispense flow rate and by positioning dispenser tips at the outer edge of the well (e.g., horizontal dispense offset towards the left side of the well). This is most important after the detection solution incubation step. See **Appendix A** for more information on plate-washing recommendations.

- When performing manual plate washing using a multi-channel pipette, plates should be washed using at least 150 µL of wash buffer per well. Excess residual volume after washing should be removed by gently tapping the plate on a paper towel.

- Do not allow plates to dry after washing steps. Solutions associated with the next assay step should be added to the plate immediately after washing.

- Remove the plate seal before reading the plate.

- Make sure that the read buffer is at room temperature when adding to the plate.

- Do not shake the plate after adding read buffer.

- To improve interplate precision, keep time intervals consistent between adding read buffer and reading the plate. 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.

- If the sample requires higher dilutions, Diluent 100 may be used in place of assay diluent.

- Avoid prolonged exposure of the S-PLEX Detect D1 reagent and detection solutions to light. Keep stocks of S-PLEX Detect D1 reagent in the dark.

- Intense light sources can affect assay performance. Plates should be protected from direct light during the plate shaking steps for optimal results.

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.

⚠️ CAUTION: IL-1β is detected at a high level in saliva. Take precautionary measures by using appropriate PPE such as gloves, face masks, lab coats, and hair covering to prevent contamination of kit reagents while running this assay.

STEP 1: ASSEMBLE

Prepare Coating Solution

MSD provides the S-PLEX Proinflammatory Panel 1 (human) Coating Solution as a 4X stock solution. Thaw the frozen vials and bring all reagents to room temperature. Vortex each vial to mix and spin down briefly before use.

Prepare the coating solution immediately before use by combining the following reagents. Vortex briefly to mix.

- ❑ 4,500 μL Diluent 100
- ❑ 1,500 μL of S-PLEX Proinflammatory Panel 1 (human) Coating Solution ◯

Coat the Plate

- ❑ Wash the uncoated plate 3 times with at least 150 μL/well of 1X MSD Wash Buffer or PBS-T. Prewashing the plate has been shown to increase signals and improve sensitivity in many assays.
- ❑ Add 50 μL of the coating solution to each well. Tap the plate gently on all sides. Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at room temperature for 1 hour.

  Note: While the coated plate is incubating, prepare the blocking solution, calibrators, and diluted samples.

Prepare Blocking Solution

Blocking solution is the assay diluent supplemented with Blocker S1 and Blocker S2 and is designed to reduce nonspecific binding in the sample matrix. MSD provides Blocker S1 and Blocker S2 as 100X stock solutions. Vortex each vial to mix and spin down briefly before use.

Prepare the blocking solution by combining the following reagents. Vortex briefly to mix.

- ❑ 3,430 μL of Diluent 58
- ❑ 35 μL of 100X Blocker S1 ⚪
- ❑ 35 μL of 100X Blocker S2 🔴

  Notes:

  - One vial each of Blocker S1 and Blocker S2 is sufficient for blocking 5 plates. If fewer than 5 plates are run, the unused Blocker S1 and Blocker S2 should be frozen immediately. The reagent is stable through 5 freeze-thaw cycles.
  - The blocking solution should be added to the plate before sample addition.

Prepare Calibrator Dilutions

MSD supplies a multianalyte lyophilized calibrator that yields the recommended highest calibrator concentration when reconstituted in 1,000 µL of Diluent 58 (Standard 1).

Prepare Standard 1:

❑ Reconstitute lyophilized S-PLEX Proinflammatory Panel 1 (human) Calibrator Blend by adding 1,000 µL of Diluent 58 to the vial. 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. Keep reconstituted calibrator on wet ice until use.

Prepare the remaining standards plus a zero standard for up to 4 replicates (Figure 2):

❑ Prepare Standard 2 by adding 50 µL of Standard 1 to 150 µL of Diluent 58. Mix by vortexing.

❑ Repeat 4-fold serial dilutions five additional times to generate Standards 3–7. Mix by vortexing between each serial dilution.

❑ Use Diluent 58 as Standard 8 (zero standard).

Note: Reconstituted calibrator is not stable when stored at 2–8 °C; however, it may be stored in aliquots at ≤–70 °C and is stable for three freeze-thaw cycles. For the lot-specific concentration of the calibrator, refer to the COA supplied with the kit. You can also find the COA at www.mesoscale.com.
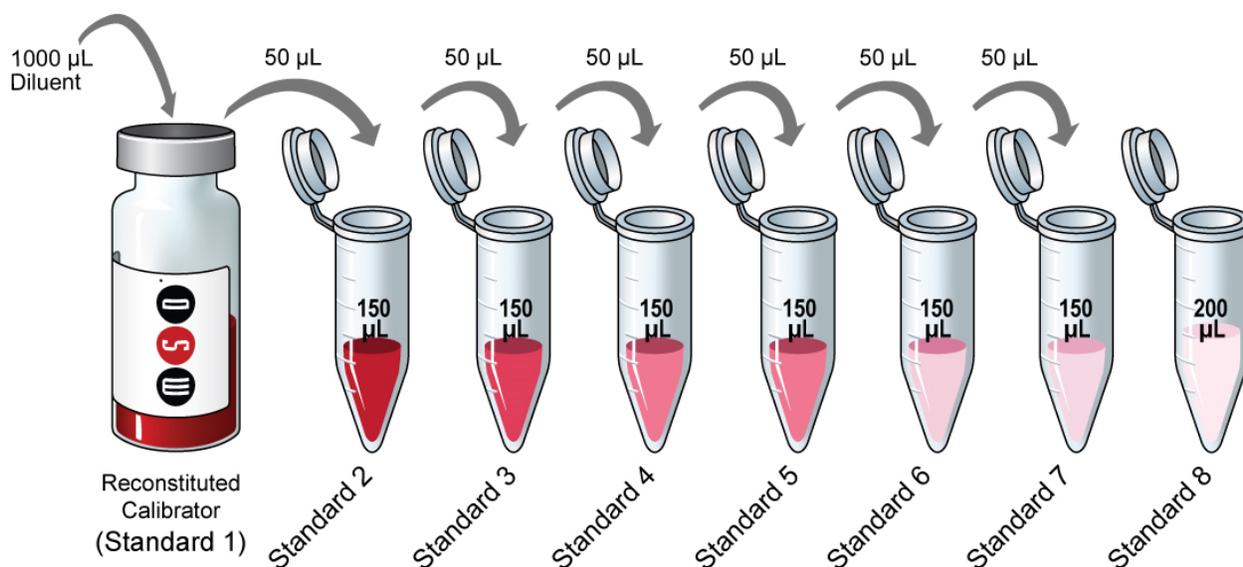
Figure 2. Dilution schema for preparation of Calibrator Standards

Sample Collection and Handling

General guidelines for sample collection, storage, and handling are presented below. If possible, use published guidelines.[1-5] 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. If there are visible particulates, centrifuge for 20 minutes at 2,000 × g before using or freezing. Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge plasma for 20 minutes at 2,000 × g within 30 minutes of collection. Use immediately or freeze.

- **CSF**: MSD recommends reviewing current literature and protocols for the collection and handling of CSF samples or the use of published guidelines.[4]
- **Other samples**. Use immediately or freeze.

Freeze all samples in suitably sized aliquots; they may be stored at ≤–10 °C until needed. Repeated freeze-thaw of samples is not recommended. After thawing, centrifuge samples at 2,000 × g for 3 minutes to remove particulates before sample preparation. Hold on wet ice or at 2–8 °C until used in the assay.

Dilute Samples

Most human samples do not require dilution. For measuring IL-6 in human CSF, MSD recommends dilutions between 10-fold and 20-fold. If dilution is needed, dilute samples with Diluent 58. For example, to dilute samples 2-fold, add 30 µL of sample to 30 µL of Diluent 58. To dilute samples 10-fold, add 10uL of sample to 90 uL of Diluent 58. The assay requires 25 µL/well of the sample. We recommend running at least two replicates per sample. You may conserve the sample by using a higher dilution. The dilution factor for other sample types will need to be optimized. The kit includes diluent sufficient enough for running samples in duplicates. Additional diluent can be purchased at www.mesoscale.com.

Add Calibrators and Sample

- ❑ After coating incubation completion, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 25 µL of blocking solution to each well. Tap the plate gently on all sides.
- ❑ Add 25 µL of calibrator or sample to each well.
- ❑ Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at room temperature for 1.5 hours.

Prepare TURBO-BOOST Antibody Solution

MSD provides each TURBO-BOOST detection antibody separately as a 200X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately before use. Bring all reagents to room temperature. Vortex each vial to mix and spin down briefly before use.

Prepare the TURBO-BOOST antibody solution by combining the following reagents. Vortex briefly to mix.

- ❑ 5,730 µL of Diluent 59
- ❑ 30 µL of TURBO-BOOST Human IFN-γ Antibody ●
- ❑ 30 µL of TURBO-BOOST Human IL–1β Antibody ●
- ❑ 30 µL of TURBO-BOOST Human IL-2 Antibody ●
- ❑ 30 µL of TURBO-BOOST Human IL-4 Antibody ●
- ❑ 30 µL of TURBO-BOOST Human IL-6 Antibody ●
- ❑ 30 µL of TURBO-BOOST Human IL-10 Antibody ●
- ❑ 30 µL of TURBO-BOOST Human IL-12p70 Antibody ●
- ❑ 30 µL of TURBO-BOOST Human IL-17A Antibody ●
- ❑ 30 µL of TURBO-BOOST Human TNF-α Antibody ●

Note: Concentrations of IL-6 in CSF have a wide range and can be orders of magnitude above the upper end of the assay range. MSD recommends excluding TURBO-BOOST IL-6 Antibody from the TURBO-BOOST antibody solution while measuring non-IL-6 assays in CSF.

Custom S-PLEX kits

For one plate, combine 30 µL of each supplied TURBO-BOOST detection antibody with enough Diluent 59 to bring the final volume to 6,000 µL.

Add TURBO-BOOST Antibody Solution

❑ After calibrator and sample incubation, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.

❑ Add 50 µL of TURBO-BOOST antibody solution to each well.

❑ Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at room temperature for 1 hour.

Note: While the TURBO-BOOST antibody solution is incubating, thaw 1 vial each of S-PLEX Enhance E1 and E2 reagents at room temperature and E3 reagent on ice.

STEP 2: ENHANCE

Prepare Enhance Solution

Prepare the enhance solution up to 30 minutes before use. Vortex each thawed vial to mix and spin down briefly before use.

Prepare enhance solution by combining the following reagents. Vortex briefly to mix.

❑ 2,970 µL Molecular Biology Grade Water

❑ 1,500 µL of 4X S-PLEX Enhance E1

❑ 1,500 µL of 4X S-PLEX Enhance E2

❑ 30 µL of 200X S-PLEX Enhance E3

Note: S-PLEX Enhance E3 stock solution is viscous. Pipette slowly to avoid bubble formation in the pipette tip and to ensure an accurate volume is pipetted.

Add Enhance Solution

❑ After TURBO-BOOST antibody incubation, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.

❑ Add 50 µL of enhance solution to each well.

❑ Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at room temperature for **30 minutes**.

Notes:

• While the enhance solution is incubating, thaw 1 vial each of S-PLEX Detect D1 at room temperature and Detect D2 on ice.

• CRITICAL: The TURBO-TAG detection incubation (next step) requires incubation at 27 °C. Upon completion of the enhance solution incubation, prepare a shaker at 27 °C. If you do not have access to a temperature-controlled shaker, a plate shaker can be placed inside an incubator maintaining 27 °C.

Prepare TURBO-TAG Detection Solution

Prepare the TURBO-TAG detection solution up to 30 minutes before use. Vortex each thawed vial to mix and spin down briefly before use.

Prepare TURBO-TAG detection solution by combining the following reagents. Vortex briefly to mix.

- ❏ 4,470 µL Molecular Biology Grade Water
- ❏ 1,500 µL of 4X S-PLEX Detect D1
- ❏ 30 µL of 200X S-PLEX Detect D2

Notes:

- CRITICAL: Avoid prolonged exposure of the S-PLEX Detect D1 reagent and the detection solution to light.
- S-PLEX Detect D2 solution is viscous. Pipette slowly to avoid bubble formation in the tip and to ensure an accurate volume is pipetted.

Add TURBO-TAG Detection Solution

- ❏ After enhance solution incubation, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❏ Add 50 µL of TURBO-TAG detection solution to each well.
- ❏ Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at 27 °C for 1 hour.

Note: CRITICAL: The incubation temperature for this step can affect the background and assay signals, thereby affecting the assay sensitivity. It is highly recommended that TURBO-TAG detection be performed at 27 °C. If you do not have access to a temperature-controlled shaker, a plate shaker can be placed inside an incubator maintaining 27 °C.

STEP 3: READ

- ❏ After TURBO-TAG detection incubation, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T using a gentle wash step.

Notes:

- CRITICAL: For this final wash step, the best results are obtained by using a low dispense flow rate and by positioning dispense tips at the outer edge of the well (e.g., horizontal dispense offset towards the left side of the wall). See **Appendix A** for more information on plate washing recommendations if using an automated plate washer.
- Do not allow plates to dry after the final wash step. Proceed to add read buffer immediately after washing the plate.

Add Read Buffer

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

- ❏ Add 150 µL of MSD GOLD Read Buffer B to each well and read on an MSD reader. Incubation in MSD GOLD Read Buffer B is not required before reading the plate.

Note: CRITICAL: Refer to the plate-instrument compatibility table (Table 3) to ensure the correct plate is read on the compatible instrument. SECTOR plates are compatible with SECTOR and QuickPlex SQ instruments.

Assay Performance

A representative data set from the development studies for the S-PLEX Proinflammatory Panel 1 (human) Kit is presented below. The kit release specifications for precision, accuracy, and sensitivity for each kit lot can be found in the lot-specific COA. The lot-specific COA is supplied with the kit and is available for download at www.mesoscale.com.

Representative Calibrator Curve
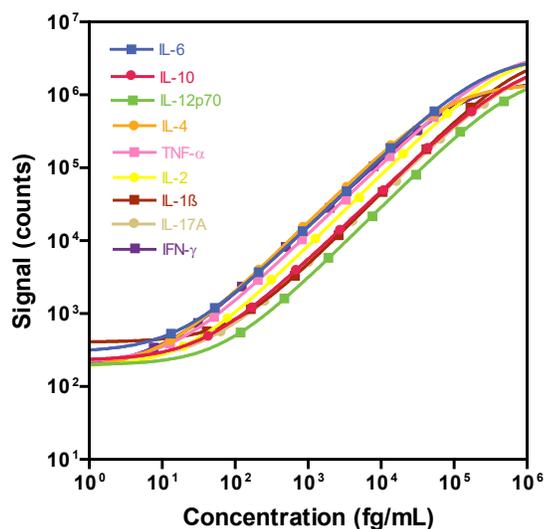
The calibration curves used to calculate analyte concentrations were established by fitting the signals from the Calibrators using a 4-parameter logistic (or sigmoidal dose-response) model with a $1/Y^2$ weighting (Figure 3). The weighting function provides a better fit of data over a wide dynamic range, particularly at the low end of the calibration curve. Analyte concentrations are determined from the electrochemiluminescence signals by back-fitting to the calibration curve. These assays have a wide dynamic range (4 logs), which allows accurate measurement of samples without the need for multiple dilutions or repeated testing.

Figure 3. Typical calibrator curves for the S-PLEX Proinflammatory Panel 1 (human) kit

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero standard). The median LLOD and range shown below (Table 4) was calculated from multiple runs (N>50 runs). The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) are verified on a lot basis using a range of sample concentrations prepared by diluting the calibrator blend. The ULOQ and LLOQ are defined as the highest and lowest concentration, respectively, at which the CV of the calculated concentration is <30% for ULOQ; <25% (<30% for IL-1β) for LLOQ, and the recovery is within 70% to 130% of the known value for ULOQ; 80% to 120% (75% to 125% for IL-1β) for LLOQ. The LLOQ and ULOQ values shown below (Table 4) were calculated from five runs using a single kit lot.

Table 4. Representative performance data for each analyte in the S-PLEX Proinflammatory Panel 1 (human) kit

	Median LLOD (fg/mL)	LLOD range (fg/mL)	LLOQ (fg/mL)	ULOQ (fg/mL)
IFN-γ	9	1–61	130	21,000
IL-1β	71	20–260	170	110,000
IL-2	12	2–30	80	53,000
IL-4	8	1–22	27	36,000
IL-6	11	1–25	54	36,000
IL-10	19	2–45	170	120,000
IL-12p70	43	8–140	490	320,000
IL-17A	44	6–130	130	170,000
TNF-α	11	1–29	53	35,000

Tested Samples

Normal Human Samples

Normal human serum, EDTA plasma, citrate plasma, heparin plasma, urine, CSF, and cell culture supernatant samples were tested without dilution. The results for each sample set are displayed below (Table 5). Median and range are calculated from samples with concentrations at or above the LLOD. Percent detected is the percentage of samples with concentration at or above the LLOD.

Table 5. Normal human samples tested in the S-PLEX Proinflammatory Panel 1 (human) kit

Sample Type	Statistics	IFN-γ	IL-1β	IL-2	IL-4	IL-6	IL-10	IL-12p70	IL-17A	TNF-α
Serum (N=37)	Median (fg/mL)	171	47	28	9	2,400	210	67	69	180
	Range (fg/mL)	37–4,400	ND–9,300	9–2,000	ND–1,100	300–81,000	36–2,500	25–1,700	13–910	54–6,000
	% Detected	100	49	100	92	100	100	100	100	100
EDTA Plasma (N=17)	Median (fg/mL)	170	70	35	6	1,600	200	61	91	160
	Range (fg/mL)	38–1,700	ND–5,900	12–2,100	ND–950	330–5,800	77–2,500	35–1,700	30–900	110–5,500
	% Detected	100	41	100	94	100	100	100	100	100
Citrate Plasma (N=17)	Median (fg/mL)	170	3,300	28	5	1,500	190	57	93	160
	Range (fg/mL)	31–1,800	ND–6,500	11–2,200	3–980	280–6,100	74–2,600	28–1,600	28–910	79–5,700
	% Detected	100	12	100	100	100	100	100	100	100
Heparin Plasma (N=17)	Median (fg/mL)	170	63	27	9	1,500	200	61	110	170
	Range (fg/mL)	36–2,100	ND–6,800	10–2,200	3–1,000	350–6,700	78–2,700	23–1,700	27–980	83–6,300
	% Detected	100	29	100	100	100	100	100	100	100
Urine (N=10)	Median (fg/mL)	ND	130	ND	3	570	ND	11	23	5
	Range (fg/mL)	NA	ND–980	NA	ND–3	36–7,800	NA	ND–11	ND–23	5–6
	% Detected	0	40	0	10	100	0	10	10	20
CSF (N=12)	Median (fg/mL)	60	590	110	59	32,000	640	220	200	670
	Range (fg/mL)	3–76,000	ND–2,500	7–5,900	ND–950	1,100–AS	11–13,000	ND–540	15–40,000	5–4,600
	% Detected	100	58	100	50	100	100	67	100	100
Cell Culture Supernatant (N=5)	Median (fg/mL)	100	1,900	370	54	AS	230	360	300	4,100
	Range (fg/mL)	18–32,000	290–3,500	85–AS	24–970	NA	51–AS	91–1,200	86–1,000	1,100–AS
	% Detected	100	100	100	100	100	100	100	100	100

AS = above Standard 1; NA = not available; ND = non-detectable

Normal Non-Human Primate Samples

Assays in the S-PLEX Proinflammatory Panel 1 (human) were tested for species cross-reactivity with non-human primates (NHP)—*Rhesus macaque* and *Cynomolgus macaque*. NHP serum, EDTA plasma, and urine samples were tested without dilution. The results for each sample set are displayed below (Table 6). Median and range are calculated from samples with concentrations at or above the LLOD. Percent detected is the percentage of samples with concentration at or above the LLOD.

Table 6. Normal NHP samples tested in the S-PLEX Proinflammatory Panel 1 (human) kit

Sample Type	Statistics	IFN-γ	IL-1β	IL-2	IL-4	IL-6	IL-10	IL-12p70	IL-17A	TNF-α
Serum (N=6)	Median (fg/mL)	19	ND	58	7	1,800	ND	ND	ND	13
	Range (fg/mL)	ND–19	NA	ND–410	ND–7	780–10,000	NA	NA	NA	ND–13
	% Detected	17	0	50	17	100	0	0	0	17
EDTA Plasma (N=6)	Median (fg/mL)	17	ND	30	13	1,300	59	78	120	61
	Range (fg/mL)	ND–17	NA	ND–44	ND–13	1,000–2,600	ND–59	ND–78	ND–180	ND–61
	% Detected	17	0	33	17	100	17	17	33	17
Urine (N=7)	Median (fg/mL)	ND	ND	ND	ND	73	ND	ND	ND	ND
	Range (fg/mL)	NA	NA	NA	NA	ND–340	NA	NA	NA	NA
	% Detected	0	0	0	0	57	0	0	0	0

NA = not available; ND = non-detectable

Parallelism

Normal human serum, EDTA plasma, citrate plasma, heparin plasma, urine, and CSF samples were diluted 2-fold, 4-fold, and 8-fold before testing. Parallelism data for IL-1β, IL-2, IL-4, IL-12p70, and IL-17A assays are reported only for the CSF samples; levels of these analytes in other matrices were below the limit of detection in diluted samples. Due to the high endogenous level of IL-6 in human CSF, CSF samples were diluted 10-fold, 40-fold, 160-fold, 640-fold, and 2,560-fold before testing. Percent recovery at each dilution level was normalized to the dilution-adjusted, neat concentration, except CSF for IL-6, which was normalized to the dilution-adjusted, 10-fold concentration (Table 7). Samples may require additional dilution with assay diluent to reduce matrix effects.

$$\% \, recovery = \frac{measured \, concentration}{expected \, concentration} \times 100$$

Table 7. Analyte percent recovery at various fold dilutions in each sample type

Sample Type	Fold Dilution	IFN-γ Average % Recovery	IFN-γ % Recovery Range	IL-6 Average % Recovery	IL-6 % Recovery Range	IL-10 Average % Recovery	IL-10 % Recovery Range	TNF-α Average % Recovery	TNF-α % Recovery Range
Serum (N=4)	Neat	100	NA	100	NA	100	NA	100	NA
	2	113	96–123	111	109–115	103	85–134	98	79–111
	4	122	115–130	117	93–134	100	91–108	98	91–108
	8	120	100–135	120	105–144	104	100–112	90	85–96
EDTA Plasma (N=4)	Neat	100	NA	100	NA	100	NA	100	NA
	2	107	99–113	104	97–114	99	91–104	99	93–105
	4	105	98–111	106	99–114	96	91–100	95	87–105
	8	96	92–103	104	90–116	86	81–98	85	77–96
Citrate Plasma (N=4)	Neat	100	NA	100	NA	100	NA	100	NA
	2	93	76–100	88	75–99	86	75–95	78	64–93
	4	99	59–122	85	64–93	80	57–91	74	45–90
	8	111	96–127	106	93–115	95	79–116	88	72–108
Heparin Plasma (N=4)	Neat	100	NA	100	NA	100	NA	100	NA
	2	106	95–122	105	91–114	95	91–99	98	90–108
	4	90	52–134	78	43–98	78	56–96	71	47–98
	8	109	77–145	101	76–117	89	65–105	82	57–102
Urine (N=4)	Neat	ND	NA	100	NA	ND	NA	ND	NA
	2	ND	NA	141	130-156	ND	NA	ND	NA
	4	ND	NA	137	127-152	ND	NA	ND	NA
	8	ND	NA	155	132-192	ND	NA	ND	NA

NA = not available; ND = non-detectable

Sample Type	Fold Dilution	IFN-γ Average % Recovery	IFN-γ % Recovery Range	IL-1β Average % Recovery	IL-1β % Recovery Range	IL-2 Average % Recovery	IL-2 % Recovery Range	IL-4 Average % Recovery	IL-4 % Recovery Range	IL-10 Average % Recovery	IL-10 % Recovery Range
CSF (N=4)	Neat	100	NA	100	NA	100	NA	100	NA	100	NA
	2	121	116–135	111	107–116	118	116–120	133	126–140	105	97–114
	4	115	90–142	78	62–94	111	104–125	114	99–135	91	76–99
	8	124	97–167	ND	NA	131	107–146	152	111–201	95	89–105

NA = not available; ND = non-detectable

Table 7. continued

Sample Type	Fold Dilution	IL-12p70		IL-17A		TNF-α	
		Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
CSF (N=4)	Neat	100	NA	100	NA	100	NA
	2	121	116–135	111	107–116	118	116–120
	4	115	90–142	78	62–94	111	104–125
	8	124	97–167	ND	NA	131	107–146

NA = not available

Sample Type	Fold Dilution	IL-6	
		Average % Recovery	% Recovery Range
CSF (N=4)	Neat	AS	NA
	10	100	NA
	40	108	106–113
	160	99	94–107
	640	87	85–91
	2,560	91	89–94

AS = above Standard 1; NA = not available

Dilution Linearity

To assess linearity, normal human serum, EDTA plasma, citrate plasma, heparin plasma, urine, and CSF samples from a commercial source, as well as cell culture media, were spiked with recombinant calibrator and diluted 2-fold, 4-fold, or 8-fold before testing. Percent recovery at each dilution level was normalized to the dilution-adjusted, neat concentration (Table 8). Samples may require additional dilution with assay diluent to reduce matrix effects.

$$\% \, recovery = \frac{measured \; concentration}{expected \; concentration} \times 100$$

Table 8. Analyte percent recovery at various fold dilutions in each sample type

Sample Type	Fold Dilution	IFN-γ Average % Recovery	IFN-γ % Recovery Range	IL-1β Average % Recovery	IL-1β % Recovery Range	IL-2 Average % Recovery	IL-2 % Recovery Range	IL-4 Average % Recovery	IL-4 % Recovery Range	IL-6 Average % Recovery	IL-6 % Recovery Range
Serum (N=4)	Neat	100	NA	100	NA	100	NA	100	NA	100	NA
	2	101	94–113	104	90–116	83	64–102	117	103–134	108	98–118
	4	97	90–102	104	93–116	82	60–106	118	108–130	113	108–118
	8	107	94–118	113	99–130	84	57–117	121	111–141	119	108–135
EDTA Plasma (N=4)	Neat	100	NA	100	NA	100	NA	100	NA	100	NA
	2	104	96–111	116	103–128	90	70–107	122	111–129	108	100–114
	4	105	96–113	117	101–134	91	63–120	131	104–152	115	110–127
	8	108	100–120	126	109–136	89	57–117	138	115–157	119	106–136
Citrate Plasma (N=4)	Neat	100	NA	100	NA	100	NA	100	NA	100	NA
	2	106	99–125	111	96–117	87	70–111	123	113–137	107	102–112
	4	99	93–103	95	82–116	83	65–105	114	100–135	109	100–117
	8	102	91–123	114	98–123	89	61–137	135	120–154	117	112–124
Heparin Plasma (N=4)	Neat	100	NA	100	NA	100	NA	100	NA	100	NA
	2	102	97–109	111	99–122	92	73–115	114	92–132	109	95–118
	4	100	88–111	110	99–117	90	70–120	116	103–127	112	101–127
	8	108	103–111	118	107–127	90	63–126	130	113–142	119	106–132
Urine (N=4)	Neat	100	NA	100	NA	100	NA	100	NA	100	NA
	2	113	101-122	117	104-125	92	90-93	134	128-139	118	110-131
	4	110	104-115	105	103-108	73	72-75	132	123-140	108	104-117
	8	103	93-116	93	90-97	65	59-69	128	122-134	105	94-115
CSF (N=4)	Neat	100	NA	100	NA	100	NA	100	NA	AS	NA
	2	118	110-130	112	108-114	94	92-96	119	113-122	AS	NA
	4	114	101-132	102	91-110	78	72-86	114	100-126	AS	NA
	8	114	102-129	117	80-192	80	66-91	122	108-138	AS	NA
RPMI (N=1)	Neat	100	NA	100	NA	100	NA	100	NA	100	NA
	2	94	NA	91	NA	70	NA	97	NA	84	NA
	4	79	NA	83	NA	61	NA	102	NA	85	NA
	8	82	NA	63	NA	54	NA	91	NA	82	NA
DMEM (N=1)	Neat	100	NA	100	NA	100	NA	100	NA	100	NA
	2	100	NA	93	NA	73	NA	103	NA	100	NA
	4	87	NA	104	NA	62	NA	97	NA	87	NA
	8	95	NA	71	NA	52	NA	88	NA	96	NA

AS = above Standard 1; NA = not applicable

Table 8. continued

Sample Type	Fold Dilution	IL-10 Average % Recovery	IL-10 % Recovery Range	IL-12p70 Average % Recovery	IL-12p70 % Recovery Range	IL-17A Average % Recovery	IL-17A % Recovery Range	TNF-α Average % Recovery	TNF-α % Recovery Range
Serum (N=4)	Neat	100	NA	100	NA	100	NA	100	NA
	2	98	86–114	86	75–97	103	91–110	100	89–106
	4	95	86–99	91	74–110	102	96–109	96	84–105
	8	99	90–111	89	69–105	110	104–117	101	86–123
EDTA Plasma (N=4)	Neat	100	NA	100	NA	100	NA	100	NA
	2	105	102–111	89	75–96	109	97–115	102	89–113
	4	106	96–113	92	84–101	111	101–119	103	82–123
	8	113	105–121	87	76–101	117	110–125	101	82–118
Citrate Plasma (N=4)	Neat	100	NA	100	NA	100	NA	100	NA
	2	102	89–113	90	72–114	108	95–122	99	92–103
	4	101	92–109	92	69–115	100	92–108	93	85–102
	8	101	88–117	81	47–128	112	106–127	97	89–106
Heparin Plasma (N=4)	Neat	100	NA	100	NA	100	NA	100	NA
	2	105	102–111	99	76–125	102	94–108	103	90–109
	4	103	91–114	97	69–111	103	98–107	99	84–111
	8	104	99–106	88	65–119	119	113–130	103	87–117
Urine (N=4)	Neat	100	NA	100	NA	100	NA	100	NA
	2	116	105- 125	106	99–116	123	109-133	104	98-107
	4	108	102-113	91	89-93	115	108-124	92	88-95
	8	101	88-109	85	84-86	107	97-114	84	78-87
CSF (N=4)	Neat	100	NA	100	NA	100	NA	100	NA
	2	112	107-122	108	98-118	112	107-116	111	108-113
	4	104	96-118	93	89-103	102	90-114	100	89-109
	8	96	85-106	82	65-105	105	87-118	109	91-135
RPMI (N=1)	Neat	100	NA	100	NA	100	NA	100	NA
	2	84	NA	84	NA	91	NA	85	NA
	4	80	NA	81	NA	91	NA	81	NA
	8	78	NA	71	NA	81	NA	74	NA
DMEM (N=1)	Neat	100	NA	100	NA	100	NA	100	NA
	2	100	NA	91	NA	95	NA	90	NA
	4	96	NA	87	NA	90	NA	83	NA
	8	92	NA	77	NA	93	NA	80	NA

NA = not applicable

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Spike Recovery

Spike recovery measurements of different sample types across the quantitative range of the assays were evaluated. Multiple individual normal human samples (serum, EDTA plasma, citrate plasma, heparin plasma, urine, and CSF) were obtained from a commercial source. These samples, along with cell culture media, were spiked with calibrators at three levels (high, mid, and low) and then diluted 2-fold. Samples may require additional dilution with assay diluent to reduce matrix effects. The average % recovery for each sample type is reported along with % recovery range (Table 9).

$$\% \; recovery = \frac{measured \; concentration}{expected \; concentration} \times 100$$

Table 9. *Spike and recovery measurement of different sample types at three spiked levels*

	Serum (N=4)		EDTA Plasma (N=4)		Citrate Plasma (N=4)		Heparin Plasma (N=4)	
	Average % Recovery	% Recovery	Average % Recovery	% Recovery	Average % Recovery	% Recovery	Average % Recovery	% Recovery
IFN-γ	76	61–91	81	55–99	85	73–97	81	69–94
IL-1β	91	48–117	85	39–102	88	45–108	94	47–133
IL-2	141	121–156	145	127–160	152	133–169	151	137–161
IL-4	78	64–93	79	69–99	81	68–96	82	70–106
IL-6	87	77–96	84	74–94	93	85–103	96	86–105
IL-10	96	85–103	91	78–101	97	87–106	98	90–105
IL-12p70	104	80–116	100	82–112	107	86–118	110	82–127
IL-17A	85	74–93	88	77–98	90	83–97	88	82–93
TNF-α	101	86–117	106	89–118	115	109–123	109	104–121

	Urine (N=4)		CSF (N=4)		RPMI (N=1)		DMEM (N=1)	
	Average % Recovery	% Recovery	Average % Recovery	% Recovery	Average % Recovery	% Recovery	Average % Recovery	% Recovery
IFN-γ	64	49–84	65	55–77	81	79–83	62	59–66
IL-1β	94	86–102	102	93–109	86	58–103	87	60–106
IL-2	140	125–165	134	120–147	145	142–151	127	123–133
IL-4	82	68–95	82	75–87	98	88–107	88	76–99
IL-6	97	85–107	AS	NA	103	99–105	97	93–102
IL-10	87	67–106	90	80–97	102	100–107	91	87–96
IL-12p70	103	83–123	86	70–99	116	109–124	114	108–117
IL-17A	100	89–111	94	88–98	108	105–112	106	103–109
TNF-α	116	100–132	106	94–118	120	119–121	117	116–118

AS = above Standard 1; NA = not available

Specificity

To assess specificity, each assay in the panel was tested individually (blended calibrator-single detection and single calibrator-blend detection). Nonspecific binding was also evaluated with additional recombinant human analytes (CD20, CD27, CD276, CD28, CTACK, CTLA-4, ENA-78, Eotaxin, Eotaxin-2, Eotaxin-3, EPO, FLT3L, Fractalkine, G-CSF, GITRL, GM-CSF, GRO-α, I-309, IFN-β, IFN-α2a, IL-12/23p40, IL-13, IL-15, IL-16, IL-17B, IL-17C, IL-17D, IL-17E/IL25, IL-17F, IL-18, IL-1α, IL-1RA, IL-21, IL-22, IL-23, IL-27, IL-29, IL-2RA, IL-3, IL-31, IL-33, IL-5, IL-7, IL-8, IL-9, IP-10, I-TAC, MCP-1, MCP-2, MCP-3, MCP-4, M-CSF, MDC, MIF, MIP-1α, MIP-1β, MIP-3β, MIP-3α, MIP-5, OX40, PD1, SDF-1α, TARC, TIGIT, TLR1, TNF-β, TPO, TRAIL, TSLP, VEGFA, YKL-40). Nonspecific binding was less than 0.5% for all assays in the kit.

Recombinant human IL-17AF heterodimer does cross-react approximately 2.5% at equimolar concentration with the IL-17A assay.

$$\% \, nonspecificity \; = \frac{nonspecific \; signal}{specific \; signal} \times 100$$

Calibration

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

MSD reference calibrators for the following analytes were evaluated against NIBSC/WHO International Standards. The ratios of NIBSC standard relative to MSD calibrator concentration in fg/mL are shown in Table 10.

WHO/NIBSC International Standard (fg/mL) = MSD Calibrator Concentration (fg/mL) x conversion factor

Where conversion factor = WHO/NIBSC: MSD concentration (fg/mL)

Table 10. Calibration of MSD Calibrator against the WHO International Standard in fg/mL Concentration

Analyte	NIBSC Catalog No*	Conversion Factor
IFN-γ	87/586	1.0
IL-1β	86/680	1.0
IL-2	86/500	1.4
IL-4	88/656	1.0
IL-6	89/548	1.0
IL-10	93/722	1.0
IL-12p70	95/544	1.0
IL-17A	12/154	1.4
TNF-α	01/420	1.9

*The NIBSC catalog numbers are the same as the WHO International biological reference preparation codes

Assay Components

Calibrators

The assay calibrator blend uses the following recombinant human proteins (Table 11).

Table 11. Recombinant human proteins used in the calibrator

Calibrator	Expression System
IFN-γ	*E. coli*
IL-1β	*E. coli*
IL-2	*E. coli*
IL-4	Hamster cell line
IL-6	*E. coli*
IL-10	*E. coli*
IL-12p70	*E. coli*
IL-17A	*E. coli*
TNF-α	*E. coli*

Antibodies

The antibody source species are described in Table 12.

Table 12. Antibody source species

Analyte	Source Species		Assay Generation
	MSD Capture Antibody	MSD Detection Antibody	
IFN-γ	Mouse Monoclonal	Mouse Monoclonal	A
IL-1β	Mouse Monoclonal	Mouse Monoclonal	A
IL-2	Mouse Monoclonal	Mouse Monoclonal	B
IL-4	Mouse Monoclonal	Mouse Monoclonal	A
IL-6	Mouse Monoclonal	Mouse Monoclonal	A
IL-10	Mouse Monoclonal	Mouse Monoclonal	A
IL-12p70	Mouse Monoclonal	Mouse Monoclonal	B
IL-17A	Mouse Monoclonal	Mouse Monoclonal	B
TNF-α	Mouse Monoclonal	Mouse Monoclonal	B

References

1. Bowen RA, et al. Impact of blood collection devices on clinical chemistry assays. Clin Biochem. 2010;43:4-25.
2. Zhou H, et al. Collection, storage, preservation, and normalization of human urinary exosomes for biomarker discovery. Kidney. 2006;69:1471-6.
3. Thomas CE, et al. Urine collection and processing for protein biomarker discovery and quantification. Cancer Epidemiol Biomarkers & Prevention. 2010;19:953-9.
4. Schoonenboom NS, et al. Effects of processing and storage conditions on amyloid beta (1-42) and tau concentrations in cerebrospinal fluid: implications for use in clinical practice. Clin Chem. 2005;51:189-95.
5. Girgrah N, et al. Purification and characterization of the P-80 glycoprotein from human brain. Biochem J. 1988;256:351-6.

Appendix A: Recommended Plate Washer Parameters

When using an automated plate washer for S-PLEX assays, the best results are obtained by using a low dispense flow rate and by positioning dispense tips at the outer edge of the well (e.g., horizontal dispense offset towards the left side of the well). Ensure that the aspiration tips are positioned at the outer edge of the well (e.g., horizontal aspirate offset toward the right side of the well) and that tips do not touch the plate bottom. This low-flow rate dispense program is recommended for washing after the detection step in S-PLEX assays; all other steps can use default wash programs. However, for convenience, plates can be washed using the low dispense flow rate program for all S-PLEX assay wash steps.

We recommend creating a new program for your automated plate washer with the optimal settings before starting your S-PLEX assay. Example settings for a typical (MSD-recommended) wash program and the S-PLEX program are shown below for a common plate washer (Biotek Model 405 LS) (Table 13). The only difference from typical wash program settings are the Aspirate Height, Dispense Rate, and Dispense X-Position.

Table 13. Parameters for customized programs on the Biotek 405 LS microplate washer

Wash Program Parameters	Typical Wash Program Settings	Recommended S-PLEX Proinflammatory Panel 1 Wash Program Settings
Plate type	96	96
CYCLES		
Wash cycles	3	3
ASPIRATION		
Aspirate Type	TOP	TOP
Travel Rate	1 (4.1% 1.0 mm/second)	1 (4.1% 1.0 mm/second)
Aspirate Delay	0500 milliseconds	0500 milliseconds
Aspirate X-Position	-35 (1.600 mm)	-35 (1.600 mm)
Aspirate Y-Position	-35 (1.600 mm)	-35 (1.600 mm)
Aspirate Height	22	24 (ensure that aspiration tips do not touch well bottom)
Secondary Aspirate?	NO	NO
DISPENSE		
Dispense Rate	05	02
Dispense Volume	0300 µL/well	0300 µL/well
Vacuum Delay Volume	0300 µL/well	0300 µL/well
Dispense X-Position	00 (0.000 mm)	-35 (1.600 mm)
Dispense Y-Position	00 (0.000 mm)	00 (0.000 mm)
Dispense Height	120 (15.245 mm)	120 (15.245 mm)
OPTS		
PRE		
Wash Pre dispense?	NO	NO
Bottom Wash?	NO	NO
MIDCYC		
Wash Shake?	NO	NO
Wash Soak?	NO	NO
Home Carrier?	NO	NO
Between Cycle Pre Dispense?	NO	NO
POST		
Final Aspirate?	YES	YES
Aspirate Type	TOP	TOP
Travel Rate	3	3
Final Aspirate Delay	0500 milliseconds	0500 milliseconds
Final Aspirate X-Position	-35 (1.600 mm)	-35 (1.600 mm)
Final Aspirate Y-Position	-35 (1.600 mm)	-35 (1.600 mm)
Final Aspirate Height	22	22
Secondary Aspirate?	YES	YES
Final Aspirate Secondary X-Position	35 (1.600 mm)	35 (1.600 mm)
Final Aspirate Secondary Y-Position	35 (1.600 mm)	35 (1.600 mm)
Final Aspirate Secondary Height	22	22

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Appendix B: Frequently Asked Questions

- **Can I extend capture, sample, or detection antibody incubation times?**

  The best practice is to follow the S-PLEX protocol as outlined in the product insert. The plate coating step should not be extended overnight.

- **Can all plate incubation steps be performed at 27 °C?**

  Yes. In our study, no changes in sensitivity and minimal signal differences were observed when all incubations were conducted at 27 °C.

- **Can the recommended plate washer program be used throughout the entire protocol?**

  Yes. However, the recommended washing program is most important after the TURBO-TAG incubation step.

- **Is it possible to store any of the working solutions after the components are mixed? If so, for how long and at what temperatures?**

  All working solutions are stable at room temperature for 30 minutes. For longer periods, they should be stored on ice. They can be stored at 2–8 °C for up to 4 hours. Equilibrate each solution to room temperature 10–15 minutes before use.

- **When should I thaw my reagents?**

  Enhance Solution: Start thawing E1 and E2 at room temperature and E3 on ice 30 minutes after the start of TURBO-BOOST antibody incubation.

  TURBO-TAG Detection Solution: Start thawing D1 at room temperature and D2 on ice right after the start of the incubation of Enhance Solution.

- **Which reagents are recommended to be stored on ice? What stocks should be stored in the dark?**

  Reagents E3 and D2 are recommended to be stored on ice (they rapidly thaw completely on ice). D1 should be treated similarly to SULFO-TAG conjugated antibodies, and prolonged light exposures should be avoided.

- **For which assay steps is molecular-grade water essential? Must it be used to prepare wash buffer?**

  Wash buffer can be prepared using deionized water. Use molecular-grade water to prepare the enhance/detect reagents.

- **Can Milli-Q water be used instead of molecular-grade water in the enhance/detect steps?**

  We recommend molecular-grade water because of its known qualities and rigorous testing. If the Milli-Q water is known to be of high quality and not contaminated, Milli-Q water can be used.

- **What volume of wash buffer is needed during plate washing?**

  We recommend at least 150 µL of wash buffer per well for each washing step. However, if an automated plate washer is used adjust the volume as per the guidance in **Appendix A**.

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Summary Protocol

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

⚠️ **CAUTION**: IL-1β is detected at a high level in saliva. Take precautionary measures by using appropriate PPE such as gloves, face masks, lab coats, and hair covering to prevent contamination of kit reagents while running this assay.

STEP 1: ASSEMBLE

Coat Plate with Biotin Antibody

❑ Prewash the plate 3 times with at least 150 μL/well of 1X MSD Wash Buffer or PBS-T.

❑ Add 50 μL of coating solution containing biotinylated capture antibody to each well. Tap the plate gently on all sides. Seal plate with an adhesive plate seal.

❑ Incubate at room temperature with shaking (700 rpm) for 1 hour.

Add Samples and Calibrators

❑ Wash the plate 3 times with at least 150 μL/well of 1X MSD Wash Buffer or PBS-T.

❑ Add 25 μL of blocking solution to each well. Tap the plate gently on all sides.

❑ Add 25 μL of calibrator or sample to each well. Seal plate with an adhesive plate seal.

❑ Incubate at room temperature with shaking (700 rpm) for 1.5 hours.

Add TURBO-BOOST Antibody Solution

❑ Wash the plate 3 times with at least 150 μL/well of 1X MSD Wash Buffer or PBS-T.

❑ Add 50 μL of TURBO-BOOST antibody solution to each well. Seal plate with an adhesive plate seal.

❑ Incubate at room temperature with shaking (700 rpm) for 1 hour.

STEP 2: ENHANCE

Add Enhance Solution

❑ Wash the plate 3 times with at least 150 μL/well of 1X MSD Wash Buffer or PBS-T.

❑ Add 50 μL of enhance solution to each well. Seal plate with an adhesive plate seal.

❑ Incubate at room temperature with shaking (700 rpm) for 30 minutes.

Add TURBO-TAG Detection Solution

❑ Wash the plate 3 times with at least 150 μL/well of 1X MSD Wash Buffer or PBS-T.

❑ Add 50 μL of TURBO-TAG detection solution to each well. Seal plate with an adhesive plate seal.

❑ Incubate at 27 °C in a temperature controlled chamber with shaking (700 rpm) for 1 hour.

STEP 3: READ

Add Read Buffer

❑ Wash the plate 3 times with at least 150 μL/well of 1X MSD Wash Buffer or PBS-T using washer program with low dispense speed. See **Appendix A** for more details.

❑ Add 150 μL of MSD GOLD Read Buffer B to each well. Read the plate on an MSD instrument. Incubation in MSD GOLD Read Buffer B is not required before reading the plate.

Catalog Numbers

Table 14. Catalog numbers associated with the S-PLEX Proinflammatory Panel 1 (human) kit

| Kit Name	SECTOR Plate		
	1-Plate Kit	5-Plate Kit	25-Plate Kit
S-PLEX Proinflammatory Panel 1 (human)	K15396S -1	K15396S -2	K15396S -4
S-PLEX Custom Human Biomarkers	K151AKV-1	K151AKV-2	K151AKV-4

Plate Diagram
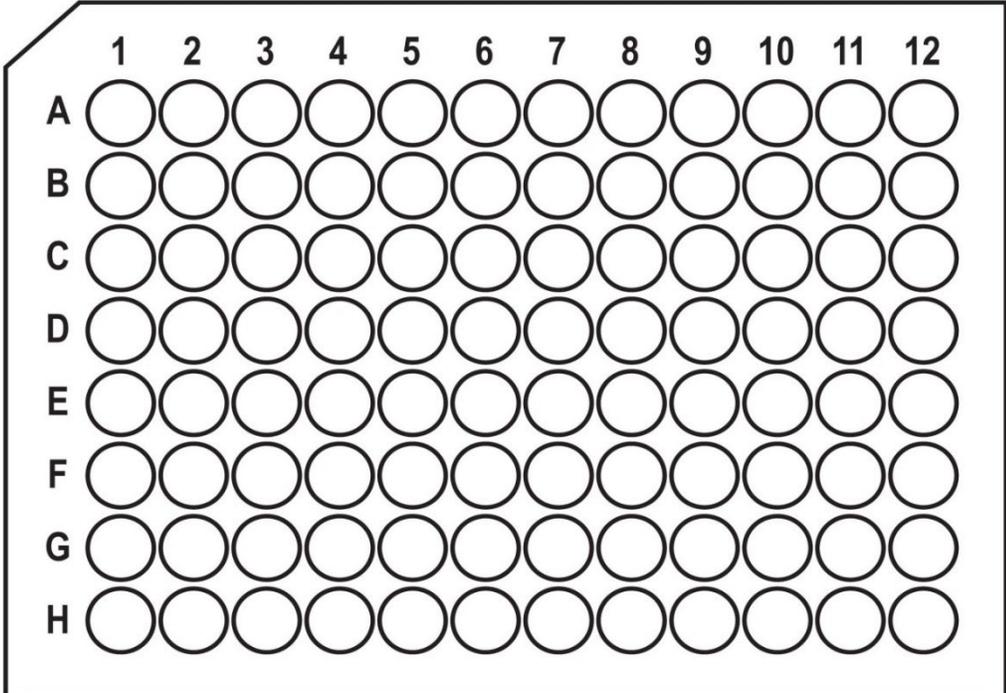
Figure 4. Plate Diagram.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL-01		Sample-01		Sample-09		Sample-17		Sample-25		Sample-33	
B	CAL-02		Sample-02		Sample-10		Sample-18		Sample-26		Sample-34	
C	CAL-03		Sample-03		Sample-11		Sample-19		Sample-27		Sample-35	
D	CAL-04		Sample-04		Sample-12		Sample-20		Sample-28		Sample-36	
E	CAL-05		Sample-05		Sample-13		Sample-21		Sample-29		Sample-37	
F	CAL-06		Sample-06		Sample-14		Sample-22		Sample-30		Sample-38	
G	CAL-07		Sample-07		Sample-15		Sample-23		Sample-31		Sample-39	
H	CAL-08		Sample-08		Sample-16		Sample-24		Sample-32		Sample-40	

Figure 5. Sample plate layout that can be used for the assay. Each sample and calibrator is measured in duplicate in side-by-side wells.