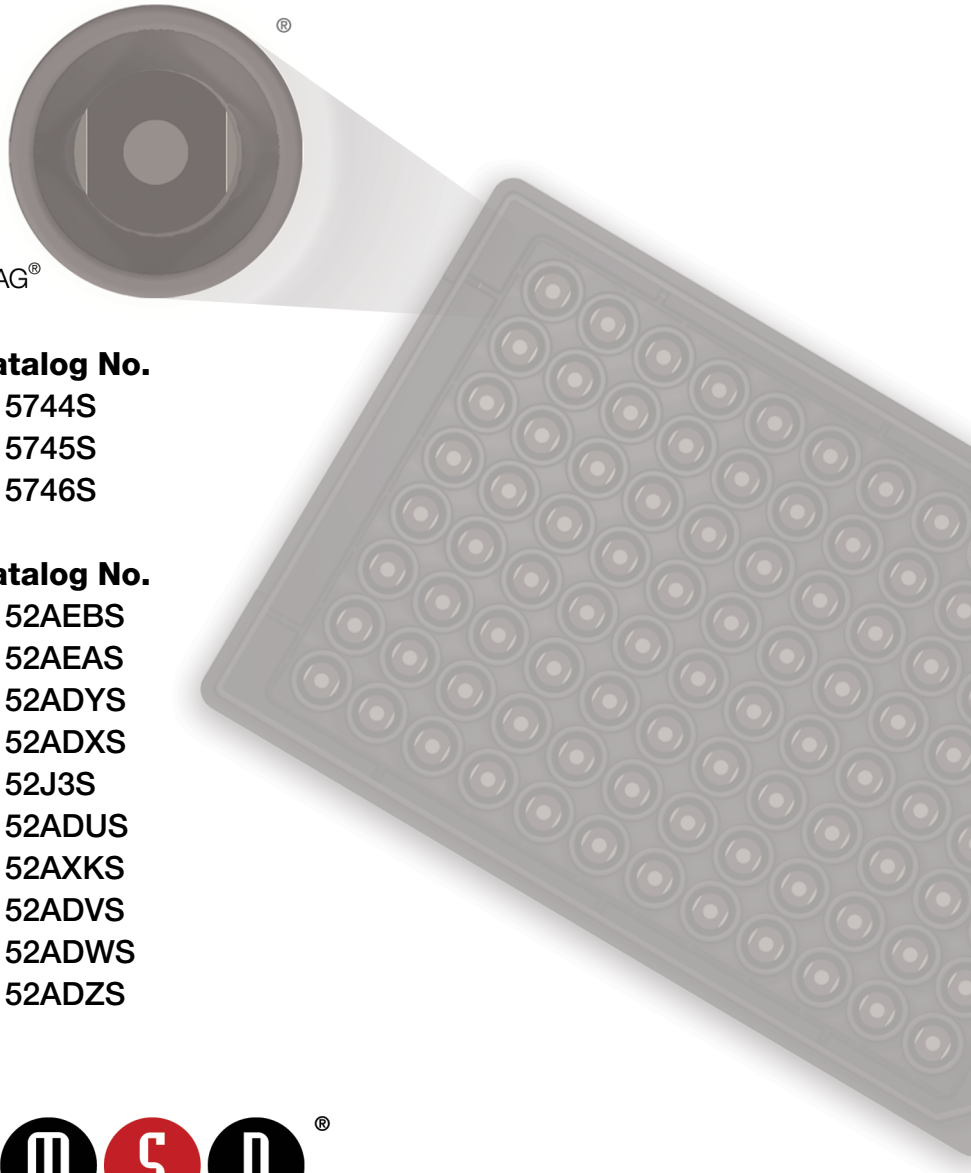
S-PLEX® Proinflammatory Panel (mouse) Kits

S-PLEX®
TrueSensitivity®
with TURBO-BOOST® & TURBO-TAG®



Multiplex Kit	Catalog No.
Proinflammatory Panel 1	K15744S
Proinflammatory Panel 2	K15745S
Proinflammatory Panel 3	K15746S

Singleplex Kit	Catalog No.
IFN-γ Kit	K152AEBS
IL-1β Kit	K152AEAS
IL-2 Kit	K152ADYS
IL-4 Kit	K152ADXS
IL-5 Kit	K152J3S
IL-6 Kit	K152ADUS
KC/GRO Kit	K152AXKS
IL-10 Kit	K152ADVS
IL-12p70 Kit	K152ADWS
TNF-α Kit	K152ADZS

MSD S-PLEX Platform

Proinflammatory Panel 1 (mouse)

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

Proinflammatory Panel 2 (mouse)

IL-1β, IL-2, IL-4, IL-5, IL-10

Proinflammatory Panel 3 (mouse)

IFN-γ, IL-6, KC/GRO, TNF-α

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

The S-PLEX Proinflammatory Panel (mouse) Kits measure cytokines that are important in inflammatory response and immune system regulation, including: Interferon-gamma (IFN-γ), Interleukin-1 beta (IL-1β), Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-5 (IL-5), Interleukin-6 (IL-6), Keratinocyte chemoattractant and growth-related oncogene (KC/GRO), Interleukin-10 (IL-10), Interleukin-12p70 (IL-12p70), and Tumor necrosis factor-alpha (TNF-α). These panels enable researchers to profile both Th1 and Th2 immune responses in mouse models, providing insights for immunology research, therapeutic efficacy studies, and safety assessments in preclinical drug development. These biomarkers are particularly valuable for evaluating immune modulation in disease models including autoimmunity, infectious disease, oncology, and inflammatory disorders.

This product insert describes the MSD S-PLEX Proinflammatory Panel (mouse) Kits, lists the components, and provides instructions for use.

S-PLEX Assay

MESO SCALE DISCOVERY® (MSD) S-PLEX assays are optimized for rapid, ultrasensitive detection of analytes from a single, small-volume sample.

S-PLEX assays deliver exceptional sensitivity that surpasses both traditional immunoassay methods and current electrochemiluminescence assays. This enhanced sensitivity opens new possibilities in biomarker research by enabling the detection and quantification of low-abundance analytes that are unmeasurable by other methods. For analytes detectable by conventional approaches, S-PLEX assays allow researchers to conserve sample volumes—a critical advantage when working with limited biological specimens.

S-PLEX technology also supports multiplexing, allowing you to measure multiple biomarkers simultaneously from a single sample. This efficiency accelerates research while maximizing the information gained from each specimen.

A true high-sensitivity assay encompasses more than just detection limits—it requires specificity. S-PLEX assays are engineered for high specificity, ensuring that the signals you detect accurately reflect your target analytes without interference from cross-reactivity or background noise. This combination of sensitivity and specificity provides the confidence and precision necessary for advancing critical discoveries in drug development and mechanistic research.

Principle of the Assay

The S-PLEX assay is a sandwich immunoassay that uses biotinylated capture antibodies and TURBO-BOOST detection antibodies (Figure 1). For singleplex assays, the biotin-labeled capture antibody binds directly to a streptavidin-coated plate. In the multiplex format, capture antibodies are pre-linked to unique MSD linkers, with each linker-coupled capture antibody attaching to a specific spot on the plate, enabling measurement of up to 10 analytes in a single well.

The first step in the assay protocol is to coat the appropriate SECTOR™ and QuickPlex® plate with the capture antibody or antibody-linker complexes. During the assay, the analyte of interest binds to the immobilized capture antibody. A TURBO-BOOST® detection antibody then binds to the captured analyte, completing the sandwich immunocomplex. After incubation, unbound detection antibody is washed away. The TURBO-BOOST detection antibody is then enhanced, allowing an electrochemiluminescent label, TURBO-TAG, to bind to the enhanced detection antibody. High affinity and high specificity antibodies ensure accurate analyte detection and enable the high sensitivity characteristic of S-PLEX assays.

Following final wash steps, read buffer is added to the plate, and the plate is loaded into an MSD instrument. An applied voltage causes the TURBO-TAG® label to emit light, generating an electrochemiluminescent signal that is significantly

stronger than formats using SULFO-TAG™ labeled detection antibodies. The instrument measures the intensity of emitted light, which is directly proportional to the amount of analyte present in the sample, providing quantitative measurement of each analyte.

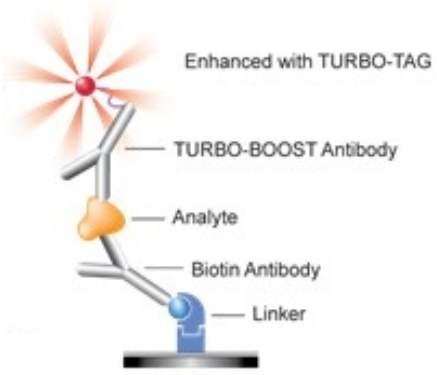

Figure 1. Principle of the S-PLEX assay.

S-PLEX Proinflammatory Panel (mouse) Kits

The capture antibodies in the S-PLEX Proinflammatory Panel (mouse) Kits locate to specific spots in each well on 10-spot MULTI-SPOT plates, as shown in the layout below (Figure 2).

Spot #	Analyte
1	IFN-γ
2	IL-1β
3	IL-2
4	IL-4
5	IL-5
6	IL-6
7	KC/GRO
8	IL-10
9	IL-12p70
10	TNF-α

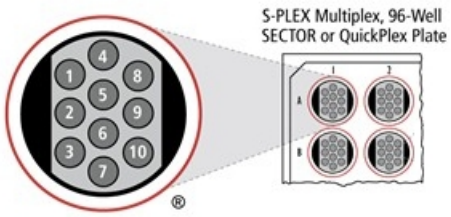

Figure 2. Spot map for the S-PLEX Proinflammatory Panel (mouse) Kits.

These assays are available in singleplex and multiplex formats. Multiplexed panels are optimized for detectability of analytes at the same dilution in tested samples and matrices. Based on normal and serum plasma levels, the three multiplex panels and their recommended starting dilution factors are:

Panel	Analytes	Dilution Factor
Proinflammatory Panel 1	IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, KC/GRO, IL-10, TNF-α	20
Proinflammatory Panel 2	IL-1β, IL-2, IL-4, IL-5, IL-10	10
Proinflammatory Panel 3	IFN-γ, IL-6, KC/GRO, TNF-α	40

The IL-12p70 assay typically requires a different dilution [neat] to the other assays and is available as a singleplex assay.

Since analyte abundance can vary in different matrices or experimental conditions, you can tailor custom multiplex panels for sample types other than normal serum and plasma. This custom panel option is available with any combination of all ten analytes.

Overview of S-PLEX Workflow

An overview of the steps in the S-PLEX workflow with incubation durations are outlined below. This is an overview for planning purposes, for the full protocol see *Protocol* on page 10.

Step	Substep	Incubation
Assemble	Coat S-PLEX plate	60 min or overnight
	Add calibrators and samples	90 min
	Add TURBO-BOOST Antibody Solution	60 min
Enhance	Add Enhance Solution	30 min
	Add TURBO-TAG Detection Solution	60 min
Read	Add Read Buffer	No incubation
	Read Plate	Read time dependent on instrument model

Materials and Equipment

Kit Components

S-PLEX assay kits are available as singleplex assays in 1-, 5-, and 25-plate sizes. See *Catalog Numbers* on page 1 for a complete list of kits.

Components are packaged by storage conditions for ease of storage and shipping.

Antibodies

Table 1. *Capture and detection antibodies that are supplied with the S-PLEX Proinflammatory Panel (mouse) Kits*

Reagent	Cap color	Storage	Catalog No.	Size	Quantity Supplied			Description
					1 Plate	5 Plates	25 Plates	
S-PLEX Proinflammatory Panel 1 (mouse) Coating Solution‡	○	≤–70 °C	C2744-2	1.7 mL	1	5	25	Blended biotinylated capture antibody coating solution
TURBO-BOOST Mouse IFN-γ Antibody	●	2–8 °C	D22AEB-2	45 µL	1	—	—	TURBO-BOOST conjugated detection antibody
			D22AEB-3	225 µL	—	1	5	
TURBO-BOOST Mouse IL-1β Antibody	●	2–8 °C	D22AEA-2	45 µL	1	—	—	TURBO-BOOST conjugated detection antibody
			D22AEA-3	225 µL	—	1	5	
TURBO-BOOST Mouse IL-2 Antibody	●	2–8 °C	D22ADY-2	45 µL	1	—	—	TURBO-BOOST conjugated detection antibody
			D22ADY-3	225 µL	—	1	5	
TURBO-BOOST Mouse IL-4 Antibody	●	2–8 °C	D22ADX-2	45 µL	1	—	—	TURBO-BOOST conjugated detection antibody
			D22ADX-3	225 µL	—	1	5	
TURBO-BOOST Mouse IL-5 Antibody	●	2–8 °C	D22J3-2	45 µL	1	—	—	TURBO-BOOST conjugated detection antibody
			D22J3-3	225 µL	—	1	5	
TURBO-BOOST Mouse IL-6 Antibody	●	2–8 °C	D22ADU-2	45 µL	1	—	—	TURBO-BOOST conjugated detection antibody
			D22ADU-3	225 µL	—	1	5	
TURBO-BOOST Mouse KC/GRO Antibody	●	2–8 °C	D22AXK-2	45 µL	1	—	—	TURBO-BOOST conjugated detection antibody
			D22AXK-3	225 µL	—	1	5	
TURBO-BOOST Mouse IL-10 Antibody	●	2–8 °C	D22ADV-2	45 µL	1	—	—	TURBO-BOOST conjugated detection antibody
			D22ADV-3	225 µL	—	1	5	
TURBO-BOOST Mouse IL-12p70 Antibody	●	2–8 °C	D22ADW-2	45 µL	1	—	—	TURBO-BOOST conjugated detection antibody
			D22ADW-3	225 µL	—	1	5	
TURBO-BOOST Mouse TNF-α Antibody	●	2–8 °C	D22ADZ-2	45 µL	1	—	—	TURBO-BOOST conjugated detection antibody
			D22ADZ-3	225 µL	—	1	5	

Dash (—) = not applicable.

Calibrator Mix

Table 2. *Calibrator mix that is supplied with the S-PLEX Proinflammatory Panel (mouse) Kits*

Reagent	Cap color	Storage	Catalog No.	Size	Quantity Supplied			Description
					1 Plate	5 Plates	25 Plates	
S-PLEX Proinflammatory Panel 1 (mouse) Calibrator Blend ‡	●	≤–70 °C	C0744-2	50 µL	1	5	25	Liquid assay calibrator

Diluents

Table 3. Diluents that are supplied with the S-PLEX Proinflammatory Panel (mouse) Kits

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1 Plate	5 Plates	25 Plates	
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	
Diluent 68	2–8 °C	R5FBB-1	10 mL	1 bottle	—	—	Assay diluent for samples and calibrator
		R5FBB-2	50 mL	—	1 bottle	5 bottles	

Dash (—) = not applicable.

Plates

Table 4. Plates that are supplied with the S-PLEX Proinflammatory Panel (mouse) Kits and their instrument compatibility

Reagent	Storage	Catalog No.	Quantity Supplied			Instrument Compatibility	Description
			1 Plate	5 Plates	25 Plates		
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
S-PLEX Multiplex 96-Well QuickPlex Plate	2–8 °C	N0B729A-1	1 plate	5 plates	25 plates	MESO QuickPlex SQ 120 MESO QuickPlex SQ 120MM MESO QuickPlex Q 60MM	Plates for coating with capture antibodies

Reagents

Table 5. Reagents that are supplied with the S-PLEX Proinflammatory Panel (mouse) Kits

Reagent	Cap color	Storage	Catalog No.	Size	Quantity Supplied			Description
					1 Plate	5 Plates	25 Plates	
Blocker S1 (100X)		≤–10 °C	R93AG-1	500 µL	1	1	5	Added to assay diluent, reduces nonspecific signals
Blocker S2 (100X)		≤–10 °C	R93AH-1	500 µL	1	1	5	Added to assay diluent, reduces nonspecific signals
S-PLEX Enhance E1 (4X)		≤–10 °C	R82AA-1	1.7 mL	1	5	25	Reagent 1 of 3 for Enhance Step
S-PLEX Enhance E2 (4X)		≤–10 °C	R82AB-1	1.7 mL	1	5	25	Reagent 2 of 3 for Enhance Step
S-PLEX Enhance E3 (200X)		≤–70 °C	R82AC-1	50 µL	1	5	25	Reagent 3 of 3 for Enhance Step
S-PLEX Detect D1 (4X)		≤–70 °C	D20K0-2	1.7 mL	1	5	25	Reagent 1 of 2 for Detection Step (contains TURBO-TAG label)
S-PLEX Detect D2 (200X)		≤–70 °C	D20J0-2	50 µL	1	5	25	Reagent 2 of 2 for Detection Step
MSD GOLD™ Read Buffer B		RT	R60AM-1	18 mL	1 bottle	—	—	Buffer to catalyze the electrochemiluminescent reaction
			R60AM-2	90 mL	—	1 bottle	5 bottles	

Lot-specific information for each assay can be found in the certificate of analysis (COA).

Assay Components Source

Calibrators

Calibrator	Expression System
IFN-γ	*E. coli*
IL-1β	*E. coli*
IL-2	*E. coli*
IL-4	*E. coli*
IL-5	*Sf 21*
IL-6	*E. coli*
KC/GRO	*E. coli*
IL-10	*E. coli*
IL-12p70	*Sf 21*
TNF-α	*E. coli*

Antibodies

Analyte	Capture Antibody	Detection Antibody	Assay Generation
IFN-γ	Mouse Monoclonal	Mouse Monoclonal	A
IL-1β	Mouse Monoclonal	Mouse Monoclonal	A
IL-2	Mouse Monoclonal	Mouse Monoclonal	A
IL-4	Mouse Monoclonal	Mouse Monoclonal	A
IL-5	Mouse Monoclonal	Mouse Monoclonal	A
IL-6	Mouse Monoclonal	Mouse Monoclonal	A
KC/GRO	Mouse Monoclonal	Mouse Monoclonal	A
IL-10	Mouse Monoclonal	Mouse Monoclonal	A
IL-12p70	Mouse Monoclonal	Mouse Monoclonal	A
TNF-α	Mouse Monoclonal	Mouse Monoclonal	A

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) diluted to 1X
- ❑ Phosphate-buffered saline (PBS) plus 0.05% Tween-20 (PBS-T)

Equipment

- ❑ Microtiter plate shaker capable of shaking at 500–1,000 rpm and maintaining a controlled temperature of 27 °C (e.g., BioSan PST-60HL-4)
- ❑ Plate-washing equipment (automated plate washer or multichannel pipette)
- ❑ Vortex mixer
- ❑ Water bath
- ❑ Microcentrifuge

Safety

Use safe laboratory practices. Wear appropriate personal protective equipment, including 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) (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

Protocol

Best Practices

Read this product insert in its entirety before use. In addition, adhere to the following best practices:

Reagent Preparation

Do Not Mix Lots	Mixing or substituting reagents from different sources or different kit lots is not recommended. Lot information is provided in the lot-specific COA.
Thaw Diluents and E1/E2/D1 at Room Temperature	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 E3 and D2 Reagents on Ice	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.

Reagent Handling

Protect Reagents from Light	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.
Prevent Cross-Contamination	To avoid cross-contamination between vials, open vials for one protocol step at a time (vial caps are color-coded). Close the cap after use. Use filtered pipette tips, and use a fresh pipette tip for each reagent addition.
Prepare in Polypropylene Tubes	Prepare calibrators and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by vortexing after each dilution.
Use Diluent 100 for High Sample Dilutions	If the sample requires higher dilutions, Diluent 100 may be used in place of assay diluent.
Avoid Bubbles During Pipetting	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	Use reverse pipetting when necessary to avoid introducing bubbles. For empty wells, pipette gently to the bottom corner. Do not touch the pipette tip on the bottom of the wells when pipetting into the MSD Plate.

Plate Handling

Plate Shaking Guidelines	Plate shaking should be vigorous, with a rotary motion between 700–1,000 rpm.
Use New Plate Seals	Use a new adhesive plate seal for all incubation steps.
Plate Washing Guidelines	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: Recommended Plate Washer Parameters* on page 28 for more information.
Multichannel Pipette Washing Guidelines	When performing manual plate washing using a multichannel pipette, wash plates with at least 150 µL of wash buffer per well. Excess residual volume after washing should be removed by tapping the plate on a paper towel.
Do Not Dry After Washing	Do not allow plates to dry after washing steps. Add solutions associated with the next assay step to the plate immediately after washing.

Plate Reading

Remove Plate Seal	Remove the plate seal before reading the plate.
Read Buffer at Room Temperature	Make sure that the read buffer is at room temperature when adding to the plate.
Do Not Shake Plate	Do not shake the plate after adding read buffer.

Sample Collection and Handling

Below are general guidelines for mouse sample collection, storage, and handling. If possible, use published guidelines.[1, 2] 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 freeze-thaw of samples is not recommended. After thawing, centrifuge samples at 2,000g for 3 minutes to remove particulates before sample preparation.

Before You Begin

Before you begin the protocol, be aware of the following:

- Bring all reagents to room temperature.
- Read *Best Practices* on page 10.
- Volumes provided for each step are sufficient for a one-plate experiment.
- A sample plate layout is shown in *Recommended Plate Layout* on page 32.

! CRITICAL

Incubation temperatures can affect assay signals and sensitivity. For optimal results, follow the recommendations provided for each step.

STEP 1: Assemble

Prepare Coating Solution

S-PLEX Proinflammatory Panel 1 (mouse) Coating Solution is provided as a 4X stock solution. Prepare the coating solution immediately before use.

❑ 1. Thaw the frozen vials and bring all reagents to room temperature.
Vortex each vial to mix and spin down briefly before use.

❑ 2. Prepare the coating solution by combining the following reagents:
☐ 4500 µL Diluent 100
☐ 1500 µL of S-PLEX Proinflammatory Panel 1 (mouse) Coating Solution
Vortex briefly to mix.

Coat the Plate

❑ 1. 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.

❑ 2. 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.
Incubate with shaking (~700 rpm) at room temperature for 1 hour.

❑ 3. While the coated plate is incubating, prepare the blocking solution, calibrators, and diluted samples.

- Alternate Protocol, Overnight Coating Incubation: plates can be incubated overnight at 2-8 °C. First shake the plate for 15 minutes at room temperature. Further shaking is not required for the overnight coating incubation step. Bring the plate to room temperature before proceeding with the next steps.

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. Blocker S1 and Blocker S2 are provided as 100X stock solutions.

❑ 1. Thaw the frozen vials and bring all reagents to room temperature.
Vortex each vial to mix and spin down briefly before use.

❑ 2. Prepare the blocking solution by combining the following reagents:
- ☐ 3,430 µL of Diluent 68
- ☐ 35 µL of 100X Blocker S1
- ☐ 35 µL of 100X Blocker S2

Vortex briefly to mix.

i One vial 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 after use. The reagent is stable through 5 freeze-thaw cycles.

Prepare Calibrator Dilutions

MSD supplies a stock liquid calibrator that is 20-fold more concentrated than the recommended highest calibrator. We recommend a 7-point calibration curve with 4-fold serial dilution steps plus a zero standard blank (Figure 3). Thaw the stock calibrator and keep it on ice, then add it to Diluent 68 at room temperature to make the calibration curve solutions.

i Discard any unused, diluted calibration solutions. 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.

Prepare the 7 standards plus a zero standard (Standard 8) for up to 4 replicates (Figure 3):

❑ 1. Prepare Standard 1 by adding 15 µL of stock calibrator to 285 µL of Diluent 68.
Mix by vortexing.

❑ 2. Prepare Standard 2 by adding 50 µL of Standard 1 to 150 µL of Diluent 68.
Mix by vortexing.
Repeat 4-fold serial dilutions five additional times to generate Standards 3–7.
Mix by vortexing between each serial dilution.

❑ 3. Use Diluent 68 as Standard 8 (zero standard).

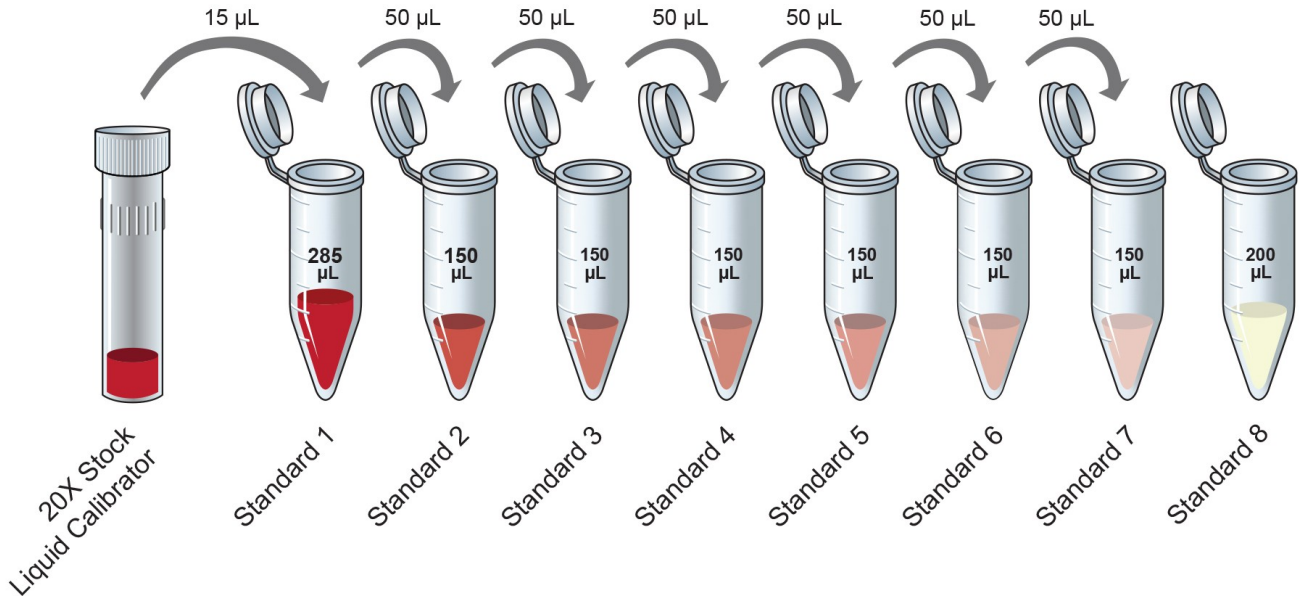

Figure 3. Dilution scheme for preparation of calibrator standards.

Dilute Samples

- See *Sample Collection and Handling* on page 1 before collecting samples.
- Dilute samples with Diluent 68.
- The assay requires 25 µL/well of diluted sample. We recommend running at least two replicates per sample.
- The kit includes diluent sufficient for running samples in duplicates. Additional diluent can be purchased at www.mesoscale.com.
- Refer to Table 6 for recommended dilutions. See *Dilution Linearity* on page 25 for more information on tested dilutions.

Table 6. Recommended starting dilutions for multiplex panels for serum and plasma.

Multiplex Panel	Recommended Dilution Factor
Proinflammatory Panel 1	20*
Proinflammatory Panel 2	10
Proinflammatory Panel 3	40

*Analytes may require different dilutions, we recommend starting at 20X. You may need to test anywhere from 5–100X depending on sample and matrix.

Add Calibrators and Sample

For a recommended plate layout, refer to Figure 6 on page 32.

- ❑ 1. If the plate was incubated overnight at 2-8 °C during the *Coat the Plate* step, equilibrate the plate by leaving the plate at room temperature for 30 minutes.
- ❑ 2. After coating incubation is complete, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ 3. Add 25 µL of blocking solution to each well.
  Tap the plate gently on all sides.
- ❑ 4. Add 25 µL of calibrator or sample to each well according to the plate layout (see also *Recommended Plate Layout* on page 32).
- ❑ 5. Seal the plate with an adhesive plate seal.
  Incubate with shaking (~700 rpm) at room temperature for 1.5 hours.

Perform this incubation step between 22 °C and 27 °C. Incubation temperatures below 22 °C for this step can negatively affect assay signals and sensitivity.

Prepare TURBO-BOOST Antibody Solution

TURBO-BOOST detection antibody is provided as a 200X stock solution. The working solution is 1X. Prepare the 1X Detection Antibody Solution immediately before use.

Multiplex Proinflammatory Panel (mouse) Kits

- ❑ 1. Bring all reagents to room temperature.
  Spin down the vial before use.
- ❑ 2. Prepare the TURBO-BOOST antibody solution by combining the following reagents:

Antibody	Panel 1	Panel 2	Panel 3
TURBO-BOOST Mouse IFN-γ Antibody	30 µL	–	30 µL
TURBO-BOOST Mouse IL-1β Antibody	30 µL	30 µL	–
TURBO-BOOST Mouse IL-2 Antibody	30 µL	30 µL	–
TURBO-BOOST Mouse IL-4 Antibody	30 µL	30 µL	–
TURBO-BOOST Mouse IL-5 Antibody	30 µL	30 µL	–
TURBO-BOOST Mouse IL-6 Antibody	30 µL	–	30 µL
TURBO-BOOST Mouse KC/GRO Antibody	30 µL	–	30 µL
TURBO-BOOST Mouse IL-10 Antibody	30 µL	30 µL	–
TURBO-BOOST Mouse TNF-α Antibody	30 µL	–	30 µL
Diluent 59	5,730 µL	5,850 µL	5,880 µL
Total	6,000 µL	6,000 µL	6,000 µL

❑ 3. Vortex briefly to mix.

Singleplex Proinflammatory Panel (mouse) Kits

❑ 1. Bring all reagents to room temperature.
Spin down the vial before use.

❑ 2. Prepare the TURBO-BOOST antibody solution by combining the following reagents:
- ☐ 5,970 µL of Diluent 59
- ☐ 30 µL of TURBO-BOOST Detection Antibody

❑ 3. Vortex briefly to mix.

Add TURBO-BOOST Antibody Solution

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

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

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

CRITICAL

Incubation temperatures below 22 °C for this step can negatively affect assay signals and sensitivity. For best results, perform this incubation between 22 °C and 27 °C.

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.

❑ 1. Thaw vials.
Vortex each thawed vial to mix and spin down briefly before use.

❑ 2. Prepare the enhance solution by combining the following reagents.
- ☐ 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

Vortex briefly to mix.

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

Add Enhance Solution

- ❑ 1. After TURBO-BOOST antibody incubation, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ 2. Add 50 µL of enhance solution to each well.
- ❑ 3. Seal the plate with an adhesive plate seal.
  Incubate with shaking (~700 rpm) at room temperature for 30 minutes.

CRITICAL

For best results, perform this incubation step between 22 °C and 27 °C.

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

Prepare TURBO-TAG Detection Solution

Prepare the TURBO-TAG detection solution up to 30 minutes before use.

- ❑ 1. 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.
- ❑ 2. Thaw vials.
  Vortex each thawed vial to mix and spin down briefly before use.

CRITICAL

Avoid prolonged exposure of the S-PLEX Detect D1 reagent and detection solution to light.

- ❑ 3. Prepare TURBO-TAG detection solution by combining the following reagents.
  - ☐ 4,470 µL Molecular Biology Grade water
  - ☐ 1,500 µL of 4X S-PLEX Detect D1
  - ☐ 30 µL of 200X S-PLEX Detect D2

  Vortex briefly to mix.

S-PLEX Detect D2 solution is viscous. Pipette slowly to avoid bubble formation in the tip and to ensure accurate pipetting volume.

Add TURBO-TAG Detection Solution

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

❑ 2. Add 50 µL of TURBO-TAG detection solution to each well.

❑ 3. Seal the plate with an adhesive plate seal.
Incubate with shaking (~700 rpm) at 27 °C for 1 hour.

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

Add Read Buffer

❑ 1. 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.
Do not allow plates to dry after the final wash step. Proceed immediately after washing the plate.

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: Recommended Plate Washer Parameters* on page 28 for more information if using an automated plate washer.

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

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

Read Plate

Refer to Table 2 on page 1 (*Instrument compatibility for each plate type*) to ensure the plate is read on a compatible instrument.

Alternate Protocols

The following alternative protocols may be considered.

IMPORTANT

The use of an alternate protocol may result in sample concentrations that vary from concentrations obtained with the standard protocol. MSD recommends using the same protocol for the entirety of a research project. Note that alternate protocols should be tested with representative samples before using for the entirety of the study.

- **Alternate Protocol, Overnight Coating Incubation**: During the coating step in *STEP 1: Assemble* on page 1, plates can be incubated overnight at 2-8 °C. First shake the plate for 15 minutes at room temperature. Further shaking is not required for the overnight coating incubation step. Bring the plate to room temperature before proceeding with the next steps.

Assay Characteristics

Assay Performance

A representative data set for the S-PLEX analyte assay is presented below (Figure 4; Table 7).The data were generated during the development of the assay using a single kit lot. 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.

Calibrator Curve and Sensitivity

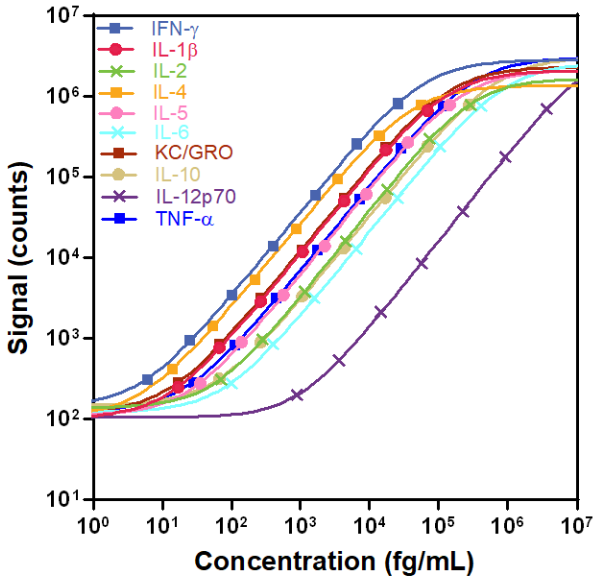

Figure 4. Typical calibrator curves for the S-PLEX Proinflammatory Panel (mouse) Kits.

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. Analyte concentrations were determined from the ECL signals by back-fitting to the calibration curve.

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 shown (Table 7) was calculated from five runs using a single kit lot. The LLOQ (lower limit of quantification) and ULOQ (upper limit of quantification) were verified on a lot basis by testing a range of samples prepared by diluting the calibrator to concentrations around the expected ULOQ and LLOQ, and assessing accuracy (70% to 130% for ULOQ and 75% to 125% for LLOQ) and precision (30% for ULOQ and 25% for LLOQ).

Table 7. LLOD and LLOQ for each analyte in the S-PLEX Proinflammatory Panel (mouse) Kits.

Analyte	LLOD (fg/mL)	LLOQ (fg/mL)	ULOQ (fg/mL)
IFN-γ	1.84	5.80	15,400
IL-1β	7.19	18.7	49,900
IL-2	17.0	34.6	185,000
IL-4	1.77	6.70	35,700
IL-5	6.52	19.4	103,000
IL-6	19.6	53.1	283,000
KC/GRO	6.19	21.2	56,500
IL-10	19.9	69.3	185,000
IL-12p70	197	429	2,290,000
TNF-α	8.22	32.5	86,700

Tested Samples

Mouse Samples

Commercially sourced mouse serum, EDTA plasma, citrate plasma, and heparin plasma were tested with indicated dilution (Table 8, Table 9, Table 10, Table 11). Medians are calculated from all tested samples. Percent detected is the percentage of samples tested with concentrations at or above the LLOD.

Table 8. Samples tested in the S-PLEX Proinflammatory Panel (mouse) Kits at 20-fold dilution.

Sample Type	Fold Dilution	Statistics	IFN-γ	IL-1β	IL-2	IL-4	IL-5	IL-6	KC/GRO	IL-10	TNF-α
Serum (n = 12)	20	Median (fg/mL)	2,579	7,506	5,107	383	4,769	159,402	197,621	17,489	58,147
		Range (fg/mL)	830 - 19,981	3,897 - 12,427	4,002 - 9,100	235 - 763	879 - 12,740	49,584 - 1,511,197	135,415 - 917,139	5,549 - 41,305	37,556 - 153,615
		% Detected	100%	100%	100%	100%	100%	100%	100%	100%	100%
EDTA Plasma (n = 12)	20	Median (fg/mL)	1,416	8,637	2,620	276	2,149	65,196	372,062	11,222	20,415
		Range (fg/mL)	374 - 3,063	2,029 - 19,996	1,571 - 4,034	153 - 514	791 - 7,283	40,286 - 706,724	199,373 - 990,532	8,303 - 17,297	11,543 - 38,167
		% Detected	100%	100%	100%	100%	100%	100%	100%	100%	100%
Citrate Plasma (n = 12)	20	Median (fg/mL)	1,940	5,008	4,340	428	8,554	81,691	134,770	19,769	33,932
		Range (fg/mL)	421 - 5,649	1,134 - 9,888	2,223 - 6,411	65 - 717	2,862 - 11,511	11,910 - 414,495	28,513 - 249,729	6,076 - 33,037	9,927 - 53,237
		% Detected	100%	100%	100%	100%	100%	100%	100%	100%	100%
Heparin Plasma (n = 12)	20	Median (fg/mL)	3,420	9,729	5,669	417	3,071	215,274	1,074,668	23,404	65,190
		Range (fg/mL)	1,081 - 183,835	4,817 - 13,312	4,495 - 9,326	222 - 669	1,836 - 26,557	51,809 - 979,433	443,637 - A.Q.	15,405 - 48,977	28,060 - 154,011
		% Detected	100%	100%	100%	100%	100%	100%	100%	100%	100%

A.Q: above quantitation limit

Table 9. Samples tested in the S-PLEX Proinflammatory Panel (mouse) Kits at 10-fold dilution.

Sample Type	Fold Dilution	Statistics	IL-1β	IL-2	IL-4	IL-5	IL-10
Serum (n = 12)	10	Median (fg/mL)	7,547	5,032	400	4,851	17,693
		Range (fg/mL)	4,076 - 12,668	3,838 - 9,407	263 - 768	840 - 10,670	5,633 - 40,796
		% Detected	100%	100%	100%	100%	100%
EDTA Plasma (n = 12)	10	Median (fg/mL)	8,418	2,498	224	2,360	11,728
		Range (fg/mL)	2,022 - 19,316	1,365 - 3,951	154 - 530	727 - 7,036	8,355 - 20,688
		% Detected	100%	100%	100%	100%	100%
Citrate Plasma (n = 12)	10	Median (fg/mL)	5,126	4,148	393	7,548	19,653
		Range (fg/mL)	1,128 - 9,520	1,988 - 6,073	127 - 715	2,798 - 11,499	6,503 - 33,025
		% Detected	100%	100%	100%	100%	100%
Heparin Plasma (n = 12)	10	Median (fg/mL)	9,706	5,536	398	3,239	23,320
		Range (fg/mL)	4,123 - 13,010	3,965 - 8,685	195 - 631	1,601 - 29,068	15,016 - 49,753
		% Detected	100%	100%	100%	100%	100%

Table 10. Samples tested in the S-PLEX Proinflammatory Panel (mouse) Kits at 40-fold dilution.

Sample Type	Fold Dilution	Statistics	IFN-γ	IL-6	KC/GRO	TNF-α
Serum (n = 12)	40	Median (fg/mL)	2,452	160,264	198,596	59,370
		Range (fg/mL)	767 - 19,613	49,105 - 1,436,158	132,071 - 871,230	37,189 - 151,881
		% Detected	100%	100%	100%	100%
EDTA Plasma (n = 12)	40	Median (fg/mL)	1,449	64,850	335,138	21,523
		Range (fg/mL)	324 - 3,345	36,041 - 715,989	194,189 - 1,010,168	11,484 - 40,207
		% Detected	100%	100%	100%	100%
Citrate Plasma (n = 12)	40	Median (fg/mL)	2,146	94,362	131,321	35,740
		Range (fg/mL)	462 - 6,056	11,117 - 407,537	29,289 - 235,110	10,191 - 53,767
		% Detected	100%	100%	100%	100%
Heparin Plasma (n = 12)	40	Median (fg/mL)	3,428	227,025	1,109,753	64,403
		Range (fg/mL)	930 - 176,511	50,886 - 898,202	431,032 - A.Q.	27,718 - 162,169
		% Detected	100%	100%	100%	100%

A.Q: above quantitation limit

Table 11. Samples tested for IL-12p70 in the S-PLEXProinflammatory Panel (mouse) Kits, neat.

Sample Type	Fold Dilution	Statistics	IL-12p70
Serum (n = 12)	Neat	Median (fg/mL)	898
		Range (fg/mL)	N.D.–1,200
		% Detectable	58%
EDTA Plasma (n = 12)	Neat	Median (fg/mL)	366
		Range (fg/mL)	N.D.–2,770
		% Detectable	50%
Citrate Plasma (n = 12)	Neat	Median (fg/mL)	693
		Range (fg/mL)	N.D.–2,980
		% Detectable	67%
Heparin Plasma (n = 12)	Neat	Median (fg/mL)	1,100
		Range (fg/mL)	N.D.–4,160
		% Detectable	92%

N.D. = non-detectable

Parallelism

Commercially sourced mouse serum, EDTA plasma, citrate plasma, and heparin plasma were tested at different dilutions.

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

Twelve samples per serum/plasma matrix were tested. The n below indicates how many samples were included in the analysis. Samples were excluded from the analysis if the calculated concentration was above ULOQ or below LLOQ, and/or calculated concentration CV exceeded 25%.

Table 12. *Percent recovery at various fold dilutions of each sample type, recovered to 10X.*

Sample Type	Fold Dilution	IL-1β			IL-2			IL-4		
		n	Average % Recovery	% Recovery Range	n	Average % Recovery	% Recovery Range	n	Average % Recovery	% Recovery Range
Serum	10	11	100	100	12	100	100	10	100	100
	20		98	90–107		102	88–115		99	78–119
	40		98	78–111		99	70–116		94	68–108
	100		96	75–118		111	76–142		—	—
	200		97	69–127		—	—		—	—
	Average		97	69–127		104	70–142		97	68–119
EDTA Plasma	10	9	100	100	11	100	100	4	100	100
	20		99	87–105		106	85–117		96	67–112
	40		98	89–110		109	87–137		90	67–114
	100		97	86–108		—	—		—	—
	200		98	70–120		—	—		—	—
	Average		98	70–120		107	85–137		93	67–114
Citrate Plasma	10	9	100	100	10	100	100	10	100	100
	20		105	88–122		103	79–115		102	83–121
	40		104	88–124		107	95–124		110	96–143
	100		99	83–115		114	89–138		—	—
	200		100	67–129		—	—		—	—
	Average		102	67–129		108	79–138		106	83–143
Heparin Plasma	10	12	100	100	12	100	100	11	100	100
	20		101	84–117		105	83–120		107	90–137
	40		98	85–117		108	75–125		107	81–130
	100		90	64–107		105	80–145		—	—
	200		98	75–129		—	—		—	—
	Average		97	64–129		106	75–145		107	81–137

Dash (—) = not applicable

Table 13. Percent recovery at various fold dilutions of each sample type, recovered to 10X.

Sample Type	Fold Dilution	IL-5			IL-10			IFN-γ		
		n	Average % Recovery	% Recovery Range	n	Average % Recovery	% Recovery Range	n	Average % Recovery	% Recovery Range
Serum	10	7	100	100	9	100	100	10	100	100
	20		102	88–119		98	91–108		98	92–104
	40		106	90–137		97	87–108		101	86–115
	100		109	87–127		95	85–113		98	75–115
	200		115	84–142		98	78–114		102	84–120
	Average		108	84–142		97	78–114		100	75–120
EDTA Plasma	10	8	100	100	12	100	100	7	100	100
	20		94	70–105		97	84–104		99	89–107
	40		101	84–113		99	91–107		103	98–107
	100		104	74–127		103	85–121		103	95–120
	200		—	—		—	—		109	100–121
	Average		100	70–127		100	84–121		103	89–121
Citrate Plasma	10	9	100	100	7	100	100	9	100	100
	20		106	90–121		101	99–105		101	91–106
	40		118	101–147		107	95–113		102	92–108
	100		115	101–135		108	99–115		103	95–111
	200		123	93–157		108	98–133		108	92–115
	Average		115	90–157		106	95–133		104	91–115
Heparin Plasma	10	10	100	100	11	100	100	10	100	100
	20		100	89–118		102	93–108		101	96–106
	40		97	85–119		104	95–112		101	93–107
	100		97	84–122		100	85–114		95	73–113
	200		—	—		105	77–128		105	87–127
	Average		98	84–122		103	77–128		101	73–127

Dash (—) = not applicable

Table 14. Percent recovery at various fold dilutions of each sample type, recovered to 10X, except KC/GRO in Heparin Plasma (recovered to 40X).

Sample Type	Fold Dilution	IL-6			KC/GRO			TNF-α		
		n	Average % Recovery	% Recovery Range	n	Average % Recovery	% Recovery Range	n	Average % Recovery	% Recovery Range
Serum	10	12	100	100	11	100	100	12	100	100
	20		98	84–110		103	98–112		103	91–111
	40		96	80–111		104	95–112		102	90–116
	100		98	71–115		102	88–114		104	84–112
	200		102	70–131		106	93–116		110	87–122
	Average		98	70–131		104	88–116		105	84–122
EDTA Plasma	10	12	100	100	9	100	100	12	100	100
	20		98	91–110		101	95–106		100	93–105
	40		97	89–112		96	88–104		103	99–109
	100		101	91–110		95	81–102		107	99–113
	200		105	89–120		98	89–107		115	106–129
	Average		100	89–120		98	81–107		106	93–129
Citrate Plasma	10	12	100	100	12	100	100	12	100	100
	20		100	90–107		101	96–108		104	99–111
	40		105	99–123		100	90–108		108	102–112
	100		107	90–128		96	91–102		109	101–114
	200		109	82–128		101	94–120		112	95–129
	Average		105	82–128		100	90–120		108	95–129
Heparin Plasma	10	12	100	100	11	—	—	12	100	100
	20		103	92–131		—	—		104	97–110
	40		102	88–125		100	100		105	95–113
	100		100	73–120		94	79–103		106	88–114
	200		105	82–135		94	77–104		111	96–122
	Average		102	73–135		94	77–104		107	88–122

Dash (—) = not applicable

Dilution Linearity

Commercially sourced mouse serum, EDTA plasma, citrate plasma, and heparin plasma were spiked with calibrator and tested at different dilutions. Samples may require additional dilution with assay diluent to reduce matrix effects.

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

Seven samples per serum/plasma matrix were tested. The n below indicates how many samples were included in analysis. Some were excluded for concentration CVs above 25% or values above ULOQ or below LLOQ.

Table 15. Recommended starting dilutions for multiplex panels for serum and plasma.

Multiplex Panel	Recommended Dilution Factor
Proinflammatory Panel 1	20*
Proinflammatory Panel 2	10
Proinflammatory Panel 3	40

*Analytes may require different dilutions, we recommend starting at 20X. You may need to test anywhere from 5–100X depending on sample and matrix.

Table 16. Percent recovery at various fold dilutions of each sample type, normalized to the dilution-adjusted 4-fold dilution concentration.

Sample Type	Fold Dilution	IL-1β			IL-2			IL-4		
		n	Recovery	Range	n	Recovery	Range	n	Recovery	Range
Serum	4	7	100	100	7	100	100	7	100	100
	8		98	95–106		103	97–106		98	87–105
	16		91	83–101		102	93–112		99	89–114
	32		95	87–103		108	98–126		103	92–118
	64		102	82–113		115	107–127		108	79–147
	Average		97	82–113		107	93–127		102	79–147
EDTA Plasma	4	5	100	100	7	100	100	7	100	100
	8		98	95–102		91	78–102		93	86–106
	16		98	94–105		97	90–105		86	78–91
	32		101	98–106		94	86–108		86	80–91
	64		99	89–108		98	91–112		87	76–97
	Average		99	89–108		95	78–112		88	76–106
Citrate Plasma	4	7	100	100	6	100	100	5	100	100
	8		97	92–105		95	91–101		99	96–102
	16		98	86–111		95	90–100		92	85–96
	32		105	100–112		97	92–103		93	78–117
	64		98	90–102		103	99–107		102	86–134
	Average		100	86–112		98	90–107		96	78–134
Heparin Plasma	4	7	100	100	7	100	100	7	100	100
	8		90	82–108		100	91–113		94	86–98
	16		85	78–96		104	92–116		92	86–98
	32		88	71–95		102	81–120		90	81–98
	64		85	70–94		112	91–128		95	91–98
	Average		86	70–108		106	81–128		93	81–98

Table 17. Percent recovery at various fold dilutions of each sample type, normalized to the dilution-adjusted 4-fold dilution concentration

Sample Type	Fold Dilution	IL-5			IL-10			IL-12p70		
		n	Recovery	Range	n	Recovery	Range	n	Recovery	Range
Serum	4	7	100	100	7	100	100	7	100	100
	8		99	92–104		96	92–99		110	96–129
	16		106	97–114		94	88–99		119	97–152
	32		114	106–120		95	87–102		—	—
	64		113	100–126		99	90–109		—	—
	Average		108	92–126		96	87–109		114	96–152
EDTA Plasma	4	6	100	100	7	100	100	7	100	100
	8		102	90–110		91	84–96		107	100–124
	16		103	92–113		86	78–92		117	108–127
	32		111	100–119		89	84–94		—	—
	64		109	102–122		93	86–100		—	—
	Average		106	90–122		90	78–100		112	100–127
Citrate Plasma	4	6	100	100	7	100	100	7	100	100
	8		96	88–106		93	89–99		109	91–129
	16		99	90–110		91	86–98		120	111–133
	32		102	92–111		90	83–97		—	—
	64		105	94–114		91	77–110		—	—
	Average		101	88–114		91	77–110		114	91–133
Heparin Plasma	4	6	100	100	7	100	100	7	100	100
	8		102	91–116		94	89–100		114	102–133
	16		108	89–128		93	88–98		121	114–137
	32		106	87–146		93	90–104		—	—
	64		—	—		99	90–105		—	—
	Average		105	87–146		95	88–105		117	102–137

Dash (—) = not applicable

Specificity

To assess specificity, all assays were tested (with blended detection) against a larger panel of analytes for nonspecific binding from mouse (6CKline/CCL21, BAFF, BCA 1/BLC, CD40, EPO, Eotaxin, GM CSF, IFN β, IL 13, IL 15, IL 16, IL 17A, IL 17A/F, IL 17C, IL 17E/IL 25, IL 17F, IL 21, IL 22, IL 23, IL 27p28/IL 30, IL 31, IL 33, IP 10, MCP 1, MCP 5/CCL12, MDC, MIP 1α, MIP 1β, MIP 2, MIP 3α, MMP 9 (total), NGAL/LCN2, RANTES, SDF 1α, TARC, TFN RI, VEGF A) Mouse/Rat (C-Peptide, Leptin and PYY (total)) and Human (Aβ38 (4G8), Aβ40 (4G8), Aβ42 (4G8), BDNF, FGF121, GFAP, Ghrelin, Insulin, NF-L and Tau (total)) analytes.

Nonspecific binding was less than 0.5%

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

Additional Information

Appendix A: Recommended Plate Washer Parameters

When using an automated plate washer for S-PLEX Assays, 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). 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 18).

Table 18. Parameters for customized programs on the Biotek 405 LS microplate washer

Wash Program Parameters		Typical Wash Program Settings	Recommended S-PLEX Singleplex 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	49
	Aspirate Y-Position	-35	00
	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	0010 µL/well
	Dispense X-Position	00 (0.000 mm)	-45
	Dispense Y-Position	00 (0.000 mm)	00
	Dispense Height	120 (15.245 mm)	120
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

Wash Program Parameters		Typical Wash Program Settings	Recommended S-PLEX Singleplex Wash Program Settings
POST	Final Aspirate?	YES	YES
	Aspirate Type	TOP	TOP
	Travel Rate	3	1 (4.1% 1.0 mm/sec)
	Final Aspirate Delay	0500 milliseconds	0500 milliseconds
	Final Aspirate X-Position	-35 (1.600 mm)	49
	Final Aspirate Y-Position	-35 (1.600 mm)	0
	Final Aspirate Height	22	24 (ensure that aspiration tips do not touch well bottom)
	Secondary Aspirate?	YES	NO
	Final Aspirate Secondary X-Position	35 (1.600 mm)	-
	Final Aspirate Secondary Y-Position	35 (1.600 mm)	-
	Final Aspirate Secondary Height	22	-

Appendix B: Frequently Asked Questions

Can I extend capture, sample, and detection antibody incubation times?

The best practice is to follow the S-PLEX protocol as outlined in the product insert. The plate coating step can be extended overnight. Once coating solution is added, store the plate overnight at 2–8 ºC without shaking. Equilibrate the plate to room temperature before proceeding with the next step.

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 mL 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: Recommended Plate Washer Parameters* on page 28.

Summary Protocol

STEP 1: ASSEMBLE

Coat Plate with Biotin Antibody

- ❑ 1. Prewash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ 2. Add 50 µL of coating solution to each well. Tap the plate gently on all sides. Seal the plate with an adhesive plate seal.
- ❑ 3. Incubate at room temperature with shaking (700 rpm) for 1 hour, or overnight without shaking at 2-8 °C.

Add Samples and Calibrators

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

Add TURBO-BOOST Antibody Solution

- ❑ 1. Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ 2. Add 50 µL of TURBO-BOOST antibody solution to each well. Seal the plate with an adhesive plate seal.
- ❑ 3. Incubate at room temperature with shaking (700 rpm) for 1 hour.

STEP 2: ENHANCE

Add Enhance Solution

- ❑ 1. Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ 2. Add 50 µL of enhance solution to each well. Seal the plate with an adhesive plate seal.
- ❑ 3. Incubate at room temperature with shaking (700 rpm) for 30 minutes.

Add TURBO-TAG Detection Solution

- ❑ 1. Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ 2. Add 50 µL of TURBO-TAG detection solution to each well. Seal the plate with an adhesive plate seal.
- ❑ 3. Incubate at 27 °C in a temperature-controlled shaker with shaking (700 rpm) for 1 hour.

STEP 3: READ

Add Read Buffer

- ❑ 1. Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T using a washer program with low dispense speed. See *Appendix A: Recommended Plate Washer Parameters* on page 28 for more details.
- ❑ 2. 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.

Read Plate

- ❑ 1. Refer to Table 2 on page 1 (*Instrument compatibility for each plate type*) to ensure the plate is read on a compatible instrument.

Plate Diagram

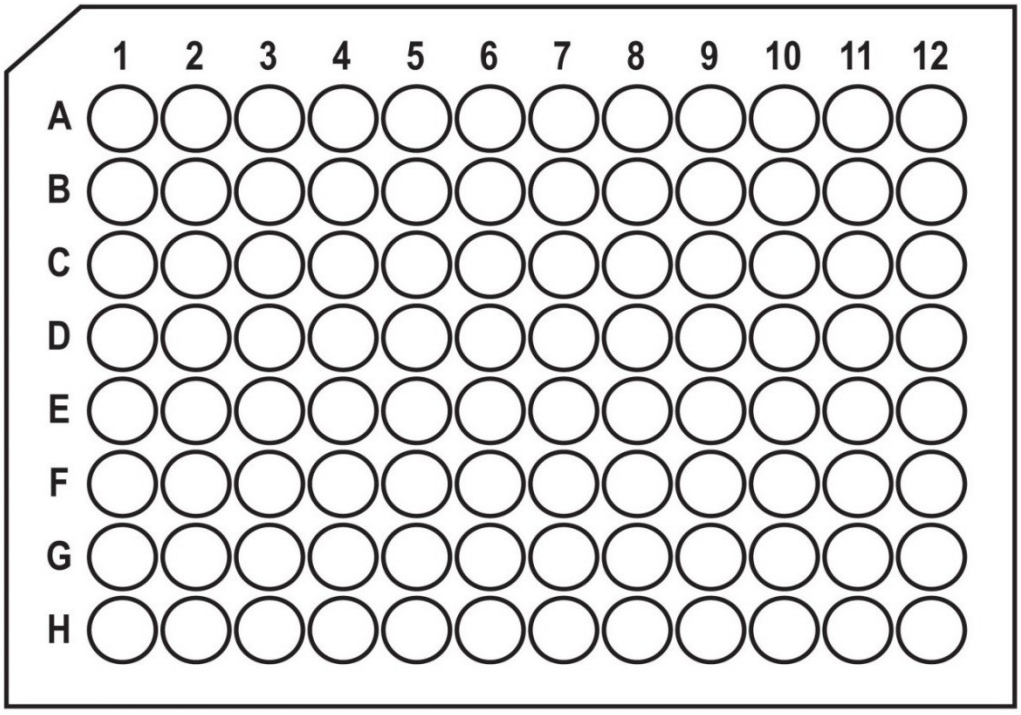

Figure 5. Plate diagram.

Recommended 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 6. Recommended plate layout for the assay. Each sample and calibrator is measured in duplicate in side-by-side wells.

Catalog Numbers

Table 19. Catalog numbers associated with the S-PLEX Proinflammatory Panel (mouse) Kits

Kit Name	SECTOR Plate			QuickPlex Plate		
	1-Plate Kit	5-Plate Kit	25-Plate Kit	1-Plate Kit	5-Plate Kit	25-Plate Kit
S-PLEX Proinflammatory Panel 1 (mouse) Kit	K15744S-1	K15744S-2	K15744S-4	K15744S-21	K15745S-22	K15744S-24
S-PLEX Proinflammatory Panel 2 (mouse) Kit	K15745S-1	K15745S-2	K15745S-4	K15745S-21	K15745S-22	K15745S-24
S-PLEX Proinflammatory Panel 3 (mouse) Kit	K15746S-1	K15746S-2	K15746S-4	K15746S-21	K15746S-22	K15746S-24
S-PLEX Mouse IFN-γ Kit	K152AEBS-1	K152AEBS-2	K152AEBS-4	K152AEBS-21	K152AEBS-22	K152AEBS-24
S-PLEX Mouse IL-1β Kit	K152AEAS-1	K152AEAS-2	K152AEAS-4	K152AEAS-21	K152AEAS-22	K152AEAS-24
S-PLEX Mouse IL-2 Kit	K152ADYS-1	K152ADYS-2	K152ADYS-4	K152ADYS-21	K152ADYS-22	K152ADYS-24
S-PLEX Mouse IL-4 Kit	K152ADXS-1	K152ADXS-2	K152ADXS-4	K152ADXS-21	K152ADXS-22	K152ADXS-24
S-PLEX Mouse IL-5 Kit	K152J3S-1	K152J3S-2	K152J3S-4	K152J3S-21	K152J3S-22	K152J3S-24
S-PLEX Mouse IL-6 Kit	K152ADUS-1	K152ADUS-2	K152ADUS-4	K152ADUS-21	K152ADUS-22	K152ADUS-24
S-PLEX Mouse KC/GRO Kit	K152AXKS-1	K152AXKS-2	K152AXKS-4	K152AXKS-21	K152AXKS-22	K152AXKS-24
S-PLEX Mouse IL-10 Kit	K152ADVS-1	K152ADVS-2	K152ADVS-4	K152ADVS-21	K152ADVS-22	K152ADVS-24
S-PLEX Mouse IL-12p70 Kit	K152ADWS-1	K152ADWS-2	K152ADWS-4	K152ADWS-21	K152ADWS-22	K152ADWS-24
S-PLEX Mouse TNF-α Kit	K152ADZS-1	K152ADZS-2	K152ADZS-4	K152ADZS-21	K152ADZS-22	K152ADZS-24

Table 20. Instrument compatibility for plate type

Plate Type	Instrument Compatibility
SECTOR™ Plate	MESO® SECTOR S 600, MESO SECTOR® S 600MM, MESO QuickPlex® SQ 120, MESO QuickPlex SQ 120MM
QuickPlex Plate	MESO QuickPlex SQ 120, MESO QuickPlex SQ 120MM, MESO QuickPlex Q 60MM

i Ensure the plate type is compatible with your MSD instrument.

References

1. Hem A, et al. Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, ferret and mink. Lab Anim. 1998;32:354-8.
2. Removal of blood from laboratory animals and birds: First report of the BVA/FRAME/RSPCA/UFAW joint working group on refinement. Lab Anim. 1993;27:1-22.