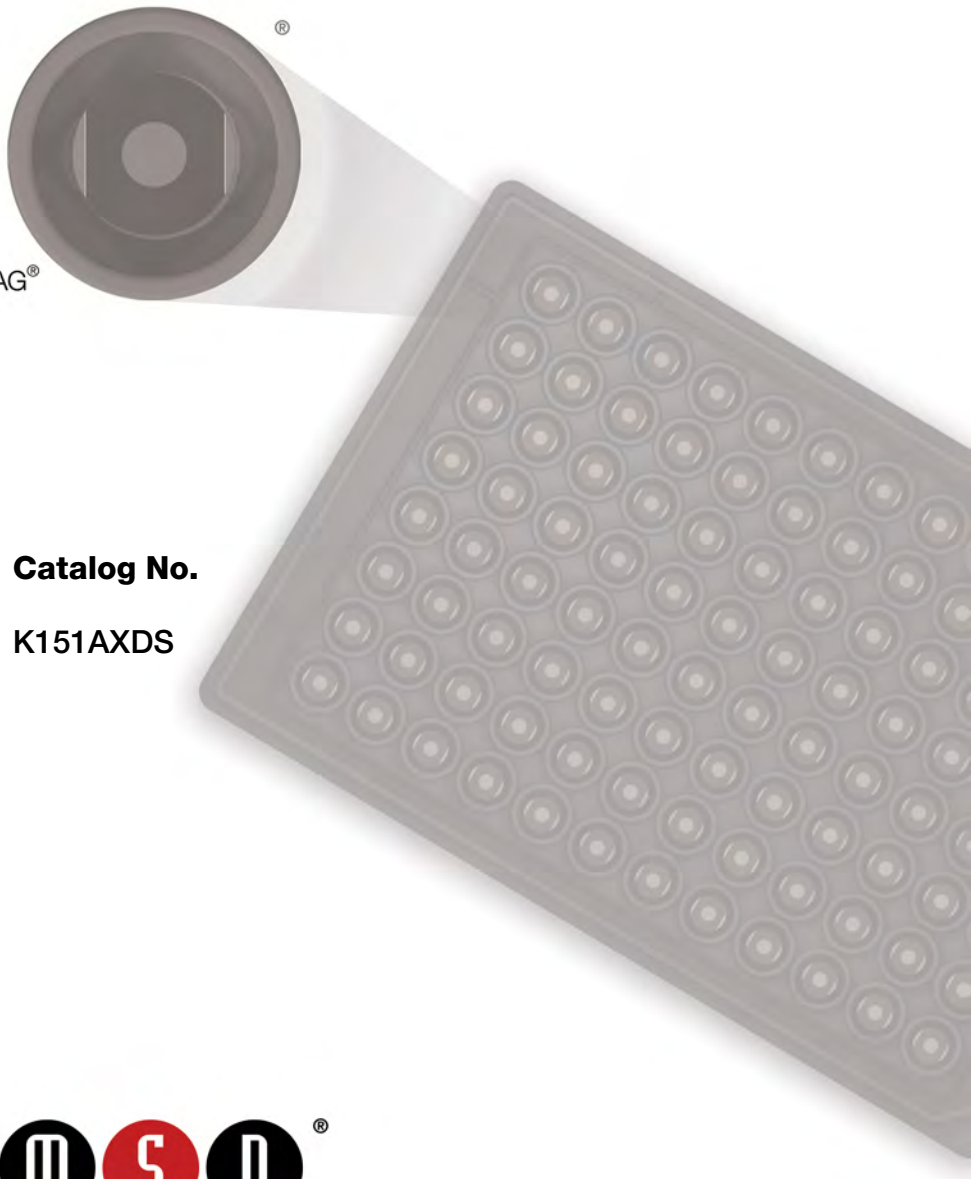
S-PLEX® IFN-ω Kit

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



	Catalog No.
S-PLEX Human IFN-ω Kit	K151AXDS

MSD S-PLEX Platform

S-PLEX Human IFN-ω Kit

For use with human serum, EDTA plasma, citrate plasma, heparin plasma, cerebrospinal fluid (CSF), and cell culture supernatants.

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

Meso Scale Discovery
A division of Meso Scale Diagnostics, LLC.
1601 Research Blvd.
Rockville, MD 20850 USA
www.mesoscale.com

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Contact Information

MSD Customer Service

Phone: 1-240-314-2795
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Technical Support

Phone: 1-240-314-2798
Fax: 1-240-632-2219 Attn: Technical Support
Email: ScientificSupport@mesoscale.com

Introduction

The MESO SCALE DISCOVERY® (MSD) **S-PLEX IFN-ω Kit** is an ultrasensitive immunoassay that measures IFN-ω protein in multiple human sample types (serum, EDTA plasma, citrate plasma, heparin plasma, and cerebrospinal fluid (CSF)).

Interferon-omega (UniProt: P05000) is a type I interferon protein involved the innate immune response to viral infections. It is a single-chain protein, composed of 195 amino acids (22.3 kDa) and can be glycosylated. IFN-ω binds to the interferon alpha/beta receptor (IFNAR) activating the JAK-STAT signaling pathway, which leads to the expression of interferon-stimulated genes (ISGs). The resulting proteins help modulate immune responses and inhibit viral replication.

Principle of the Assay

S-PLEX is MSD's ultrasensitive platform. It can dramatically improve the sensitivity of immunoassays, thus reducing the lower limit of detection (LLOD) by 10- to 1,000-fold over other assay methods. Results vary from assay to assay, but detection limits in the low femtogram/mL range are common. The reduced detection limits enable the measurement of analytes at lower concentrations and minimizes the required volumes of samples and critical reagents.

S-PLEX uses electrochemiluminescence (ECL) technology, retaining its well-known advantages and superior analytical performance. The improved sensitivity of S-PLEX is achieved with the TURBO-TAG and TURBO-BOOST reagents. When TURBO-TAG is combined with an antibody labeled with TURBO-BOOST, more ECL signal is generated than with other formats that use SULFO-TAG™ as the detection label. The S-PLEX platform uses the same robust MSD instruments as other MSD assays.

S-PLEX assays use S-PLEX 96-well SECTOR™ and QuickPlex® plates (Figure 1) that are coated with streptavidin. These plates provide high sensitivity, consistent performance, and excellent inter- and intra-lot precision. S-PLEX Kits are supplied with a biotinylated capture antibody, a TURBO-BOOST conjugated detection antibody, a calibrator, assay and antibody diluents, and S-PLEX specific reagents.

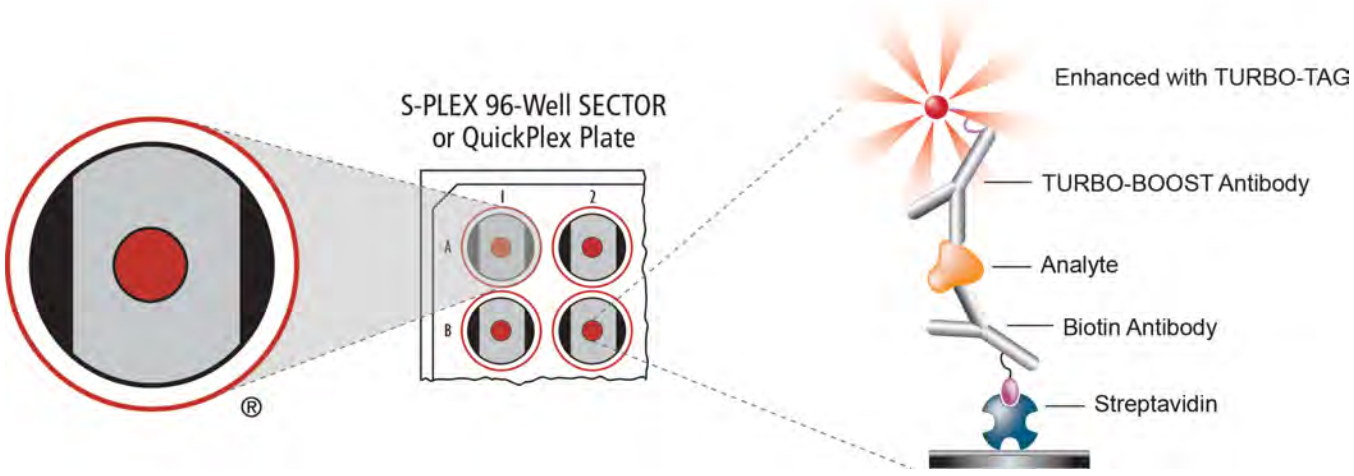

Figure 1. *S-PLEX Singleplex Assay on an S-PLEX plate.*

Performing an S-PLEX assay is similar to other MSD assays. The protocol is simple, robust, and uses common laboratory techniques. The steps involved in the formation of the detection complex are depicted in Figure 2.

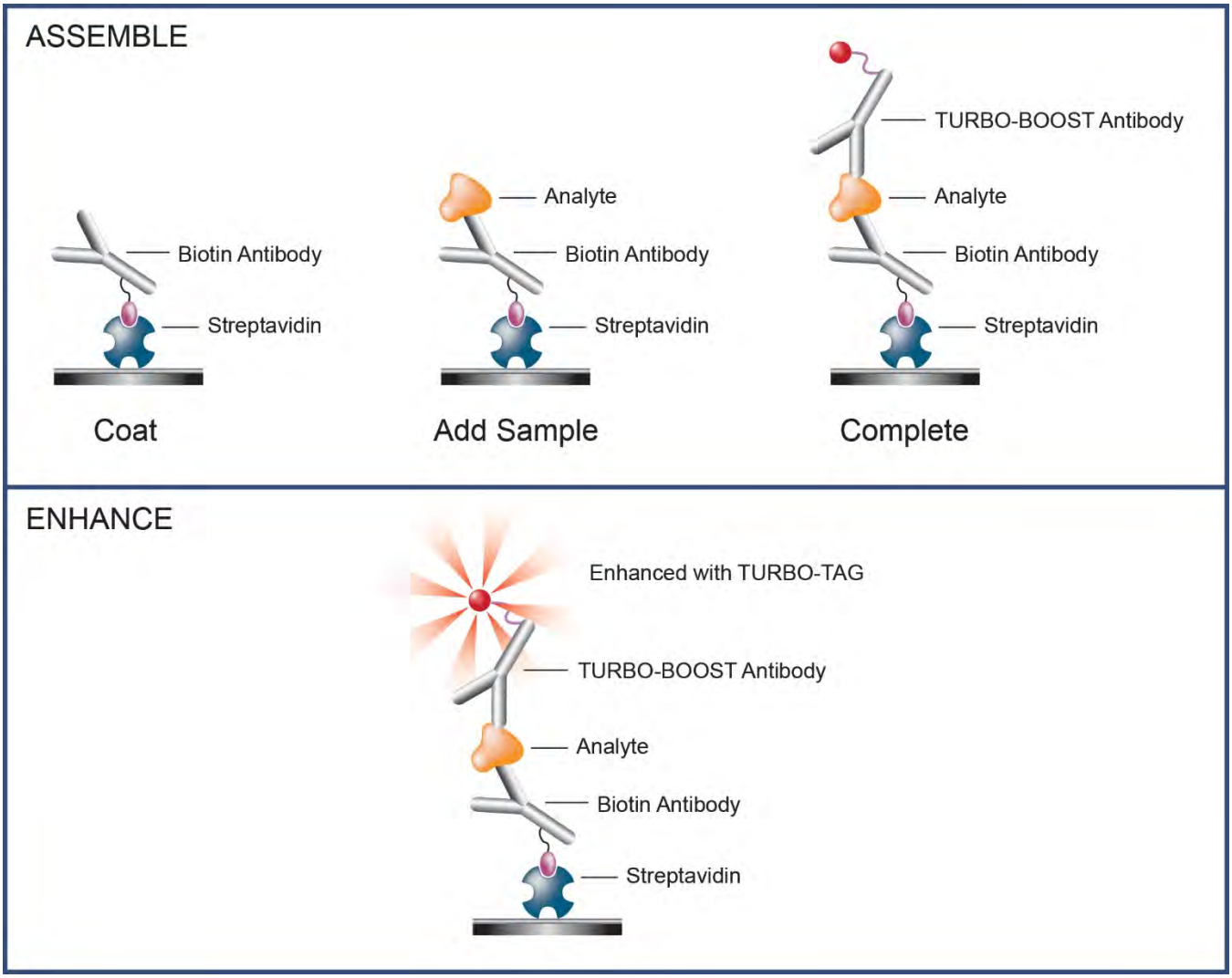

Figure 2. Formation of the S-PLEX detection complex.

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 26 for a complete list of kits.

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

Kit Lot-Specific Reagents and Components

Table 1. *Kit lot-specific reagents and components that are supplied with the S-PLEX IFN-ω Kit*

Reagent	Cap color	Storage	Catalog No.	Size	Quantity Supplied			Description
					1 Plate	5 Plates	25 Plates	
Biotin Human IFN-ω Antibody‡	○	2–8 °C	C21AXD-2	170 µL	1	—	—	Assay-specific biotinylated capture antibody
			C21AXD-3	850 µL	—	1	5	
TURBO-BOOST Human IFN-ω Antibody‡	●	2–8 °C	D21AXD-2	45 µL	1	—	—	TURBO-BOOST conjugated detection antibody
			D21AXD-3	225 µL	—	1	5	
Human IFN-ω Calibrator (20X)	●	≤–70 °C	C01AXD-2	50 µL	1 vial	5 vials	25 vials	Liquid assay calibrator
S-PLEX Coating Reagent C1 (200X)	●	≤–70 °C	C20H0-3	300 µL	1	1	5	Reagent mixed with capture antibody for plate coating, enhances assay signals
Blocker S1 (100X)	●	≤–10 °C	R93AG-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

Lot-specific information for each assay can be found in the certificate of analysis (COA).
Dash (—) = not applicable.
‡ = Biotin and TURBO-BOOST antibodies are shipped as an Antibody Set (Catalog Nos. B21AXD-2 for 1-plate and B21AXD-3 for 5- and 25-plate sizes).

Non-Kit Lot-Specific Reagents and Components

Table 2. Non-kit lot-specific reagents that are supplied with the S-PLEX IFN-ω Kit

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 66	2–8 °C	R5EBB-1	10 mL	1 bottle	—	—	Assay diluent for samples and calibrator
		R5EBB-2	50 mL	—	1 bottle	5 bottles	
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	

RT = room temperature.
Dash (—) = not applicable.

Table 3. Plates that are supplied with the S-PLEX IFN-ω Kit and their instrument compatibility

Reagent	Storage	Catalog No.	Quantity Supplied			Instrument Compatibility	Description
			1 Plate	5 Plates	25 Plates		
S-PLEX 96-Well Small Spot SECTOR Plate	2–8 °C	L45KA-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 96-Well Small Spot QuickPlex Plate	2–8 °C	L4BNA-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

Assay Components Source

Calibrators

IFN-ω protein expressed in a hamster cell line is used as a calibrator for the S-PLEX IFN-ω Kit.

Antibodies

The antibody source species are described in Table 4.

Table 4. Antibody source species

Analyte	Capture Antibody	Detection Antibody	Assay Generation
IFN-ω	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 to include 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 using this product. 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 the introduction of 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

Protect Plate from Light	Intense light sources can affect assay performance. Plates should be protected from direct light using aluminum foil during the plate shaking steps for optimal results.
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 21 for more information.
Multichannel Pipette Washing Guidelines	When performing manual plate washing using a multichannel pipette, plates should be washed using 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. Solutions associated with the next assay step should be added 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

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 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.

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 25.

! 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

Biotin Human IFN-ω Antibody is provided as a 40X stock solution and S-PLEX Coating Reagent C1 as a 200X 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:
☐ 5,820 µL Diluent 100
☐ 150 µL of Biotin Human IFN-ω Antibody
☐ 30 µL of 200X S-PLEX Coating Reagent C1 ●
Vortex briefly to mix.

❑ 3. Freeze unused S-PLEX Coating Reagent C1 immediately after use. The reagent is stable through 5 freeze-thaw cycles.

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.

Prepare Blocking Solution

Blocker S1 is provided as a 100X stock solution.

❑ 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:
- ☐ 3,465 µL of Diluent 66
- ☐ 35 µL of 100X Blocker S1

Vortex briefly to mix.

> **i** One vial of Blocker S1 is sufficient for blocking 5 plates. If fewer than 5 plates are run, the unused Blocker S1 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 and a zero standard blank (Figure 3). Thaw the stock calibrator and keep it on ice, then add it to Diluent 66 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 66.
Mix by vortexing.

❑ 2. Prepare Standard 2 by adding 50 µL of Standard 1 to 150 µL of Diluent 66.
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 66 as Standard 8 (zero standard).

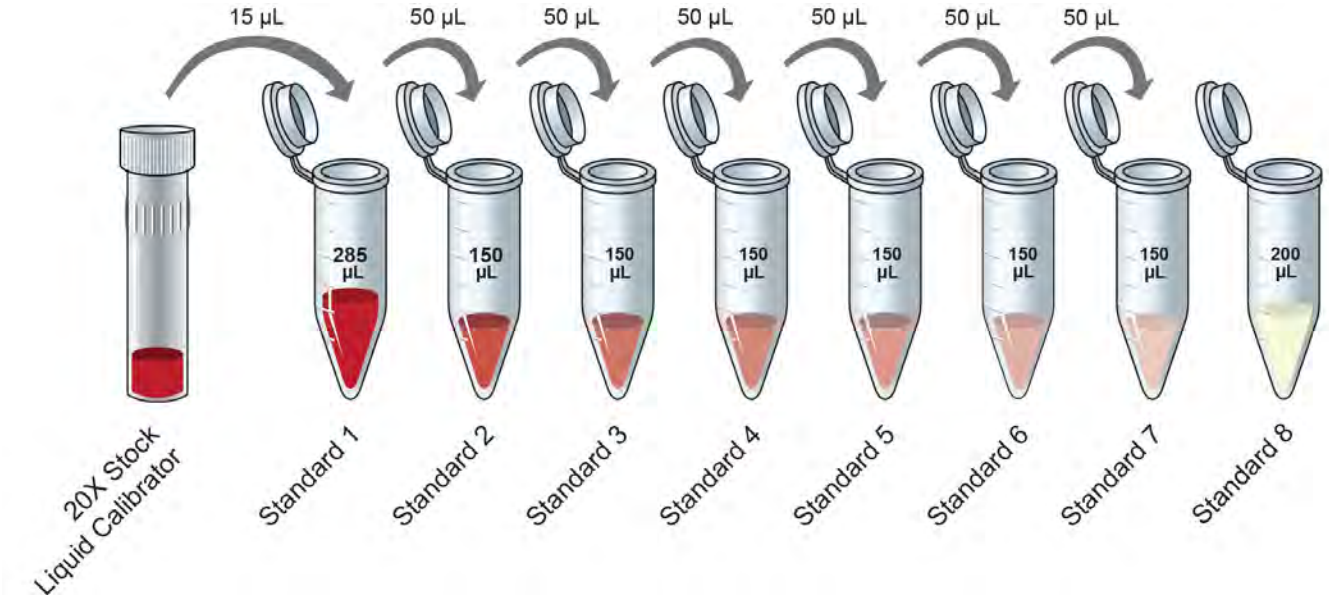

Figure 3. Dilution scheme for preparation of calibrator standards.

Dilute Samples

- See *Sample Collection and Handling* on page 11 before collecting samples.
- CSF samples require a 2X dilution for measuring IFN-ω.
- Human samples for all other sample matrices listed do not require dilution for measuring IFN-ω.
- The dilution factors for other sample types need to be optimized.
- Samples may be diluted in Diluent 66 for sample conservation.
- The assay requires 25 µL/well of 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.

Add Calibrators and Sample

For a recommended plate layout, refer to Figure 6 on page 25.

❑ 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 25).

❑ 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.

❑ 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 Human IFN-ω Antibody
Vortex briefly to mix.

Add TURBO-BOOST Antibody Solution

❑ 1. After calibrator and sample 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-BOOST antibody solution to each well.

❑ 3. 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 21 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 13 on page 26 (*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 12, 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 IFN-ω assay is presented below (Figure 4; Table 5). The data represent the performance of the assay tested in a singleplex format. 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

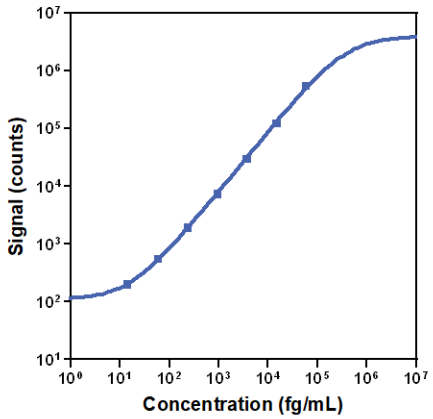

Figure 4. Typical calibrator curve for the S-PLEX IFN-ω Kit.

Metric	Value/Range
LLOD (fg/mL)	1.83
LLOD Range (fg/mL)	1.70–2.89
LLOQ (fg/mL)	27.5
ULOQ (fg/mL)	39,000

Table 5. LLOD and LLOQ for the S-PLEX IFN-ω Kit.

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 and range shown above (Table 5) were 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 80% to 120% for LLOQ) and precision (30% for ULOQ and 20% for LLOQ). The LLOQ and ULOQ shown above (Table 5) were calculated from five runs using a single kit lot.

Tested Samples

Human Samples

Cell culture supernatants and commercially sourced serum and CSF samples from individuals with disorders as well as non-diseased human serum, EDTA plasma, citrate plasma, and heparin plasma were tested without dilution. Percent detected is the percentage of samples tested with concentrations at or above the LLOD. Median and range are calculated from samples with concentrations at or above the LLOD and below ULOQ with concentration CV < 20%.

Table 6. Samples tested in the S-PLEX Human IFN-ω Kit

Sample Type	Fold Dilution	Median (fg/mL)	Range (fg/mL)	% Detected
Serum (n = 20)	Neat	2.71	N.D.–15.0	35%
EDTA Plasma (n = 20)	Neat	2.79	N.D.–14.0	40%
Citrate Plasma (n = 14)	Neat	7.97	N.D.–9.00	14%
Heparin Plasma (n = 14)	Neat	4.53	N.D.–34.0	43%
CSF (n = 10)	Neat	3.36	N.D.–5.00	40%
Serum (COVID-19) (n = 21)	Neat	3.76	N.D.–772	57%
Serum (Rheumatoid Arthritis) (n = 5)	Neat	56.2	N.D.–88.0	40%
Serum (Systemic Lupus Erythematosus) (n = 10)	Neat	118	N.D.–385	80%
EDTA Plasma (Rheumatoid Arthritis) (n = 5)	Neat	2.82	N.D.–3.00	20%
Cell Culture Supernatant (n = 8)	Neat	55.1	N.D.–3,240	63%

N.D. = non-detectable

Dilution Linearity

Commercially sourced human serum, EDTA plasma, citrate plasma, heparin plasma, and CSF samples were spiked with calibrator and tested at different dilutions. Percent recovery at each dilution level was normalized to the dilution-adjusted, neat concentration. 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 of each sample type

Sample Type	Fold Dilution	Average % Recovery	% Recovery Range
Serum (N = 12)	Neat	100	—
	2	112	105–118
	4	116	109–125
	8	121	112–130
	Average	116	105–130
EDTA Plasma (N = 12)	Neat	100	—
	2	109	104–117
	4	113	107–119
	8	116	108–127
	Average	113	104–127
Citrate Plasma (N = 6)	Neat	100	—
	2	108	106–111
	4	110	106–114
	8	111	108–117
	Average	110	106–117
Heparin Plasma (N = 6)	Neat	100	—
	2	107	102–110
	4	110	103–114
	8	111	108–115
	Average	110	102–115

Sample Type	Fold Dilution	Average % Recovery	% Recovery Range
CSF (N = 5)	Neat	100	—
	2	188	159–230
	4	209	167–273
	8	221	172–299
	Average	206	159–299

Dash (—) = not applicable.

Table 8. Analyte percent recovery at various fold dilutions of CSF, normalized to 2x

Sample Type	Fold Dilution	Average % Recovery	% Recovery Range
CSF (N = 5)	2	100	—
	4	110	103–119
	8	116	106–130
	Average	113	103–130

Dash (—) = not applicable.

Spike Recovery

Commercially sourced human serum, EDTA plasma, citrate plasma, heparin plasma, and CSF samples were spiked with calibrator at 3 levels. Samples may require additional dilution with assay diluent to reduce matrix effects.

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

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

	Average % Recovery	% Recovery Range
Serum (N=11)	86	79–99
EDTA Plasma (N=12)	86	78–103
Citrate Plasma (N=6)	83	64–90
Heparin Plasma (N=6)	86	79–94
CSF (N=5)	53	37–66

Table 10. Spike and recovery measurement of CSF with 2x dilution after spiking

	Average % Recovery	% Recovery Range
CSF (N=5)	85	76–95

Specificity

To assess specificity, the S-PLEX IFN-ω Kit was tested against a larger panel of human analytes for nonspecific binding (IL-1a, IL-1b, IL-1RA, IL-2, IL-2Ra, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12/23p40, IL-12p70, IL-15, IL-16, IL-17A, IL-17A/F, IL-17B, IL-17C, IL-17D, IL-17E/IL-25, IL-17F, IL-18, IL-21, IL-22, IL-23, IL-27, IL-29, IL-31, IL-33, TNF-α, TNF-β, IFN-γ, IFN-α2a, IFN-β, GM-CSF, G-CSF, M-CSF, VEGFa, TPO, EPO, FLT3L, TSLP, TRAIL, TARC, YKL-40, IP-10, MDC, MIF, GRO-α, I-309, Eotaxin, Eotaxin-2, Eotaxin-3, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1α, MIP-1β, MIP-3α, MIP-5).

Nonspecific binding was not detected.

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

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 11). The only differences from typical wash program settings are the Dispense Rate and Dispense X-Position.

Table 11. 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 21.

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 containing biotinylated capture antibody and Coating Reagent C1 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 21 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 13 on page 26 (*Instrument compatibility for each plate type*) to ensure the plate is read on a compatible instrument.

Plate Diagram

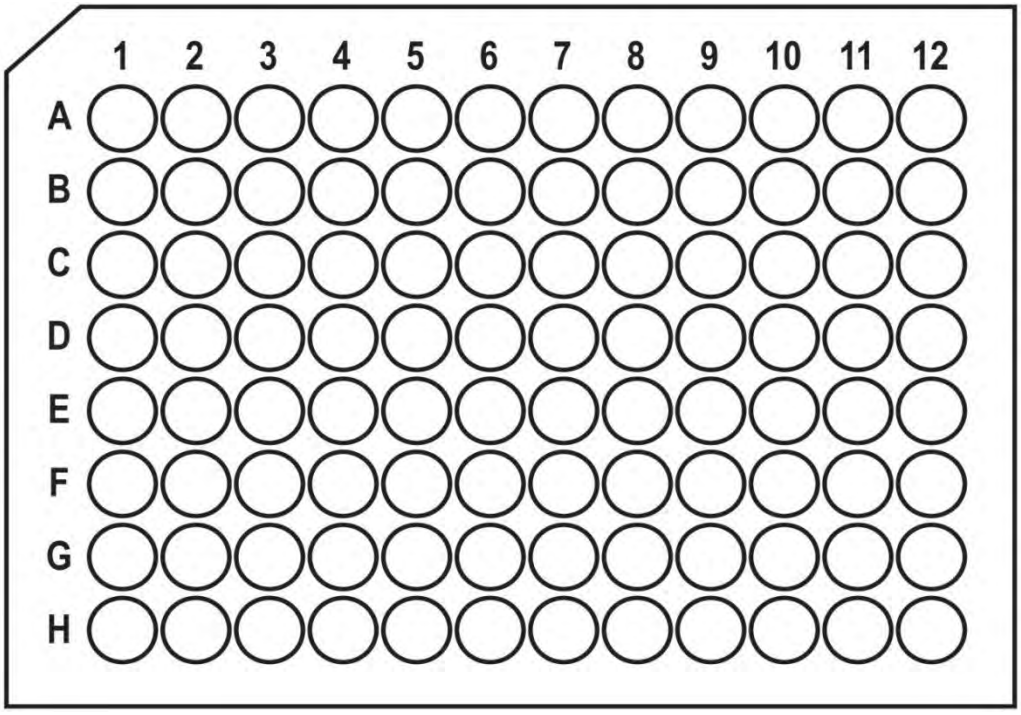

Figure 5. Plate diagram.

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 12. Catalog numbers associated with the S-PLEX IFN-ω Kit

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 Human IFN-ω	K151AXDS-1	K151AXDS-2	K151AXDS-4	K151AXDS-21	K151AXDS-22	K151AXDS-24

Table 13. Instrument compatibility for each 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

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