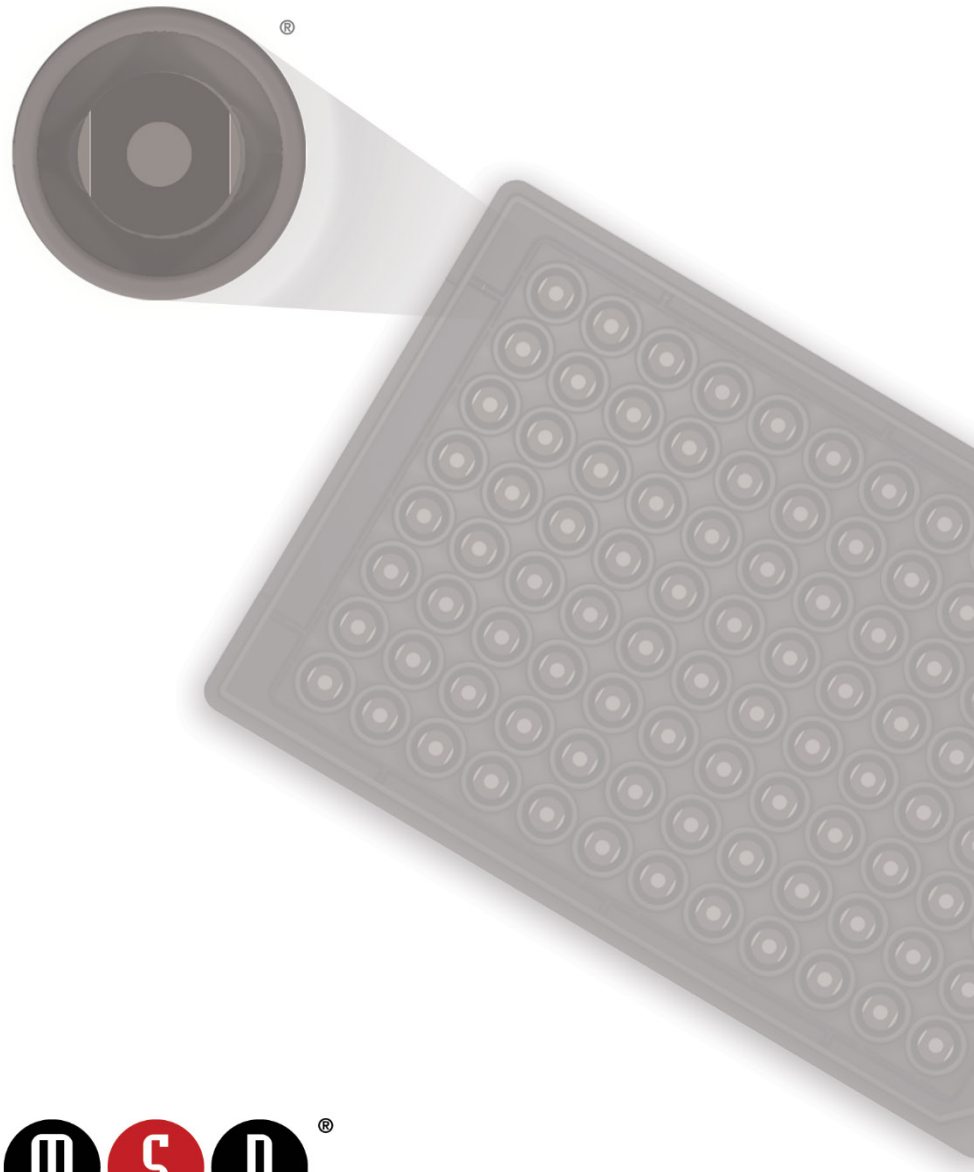
MSD® S-PLEX Platform

Human IFN-γ Kit

S-PLEX® TrueSensitivity™



	S-PLEX®
Human IFN-γ Kit	K151X9S

MSD S-PLEX Platform

S-PLEX Human IFN-γ Kit

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

Instrument Supported:

- SECTOR™ plates for use on MESO® SECTOR S 600, MESO SECTOR® S 600MM, MESO QuickPlex® SQ 120, and MESO QuickPlex SQ 120MM instrument
- QuickPlex® plates for use on MESO QuickPlex Q 60MM instrument

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

The **S-PLEX Human IFN-γ** Kit is an ultra-sensitive immunoassay developed using the S-PLEX platform. The assay measures Interferon gamma in human serum, EDTA plasma, citrate plasma, heparin plasma, CSF, and cell culture supernatant samples.

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

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

Principle of the Assay

S-PLEX assays use either S-PLEX 96-Well SECTOR or 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, calibrator, assay and antibody diluents, and S-PLEX specific reagents.

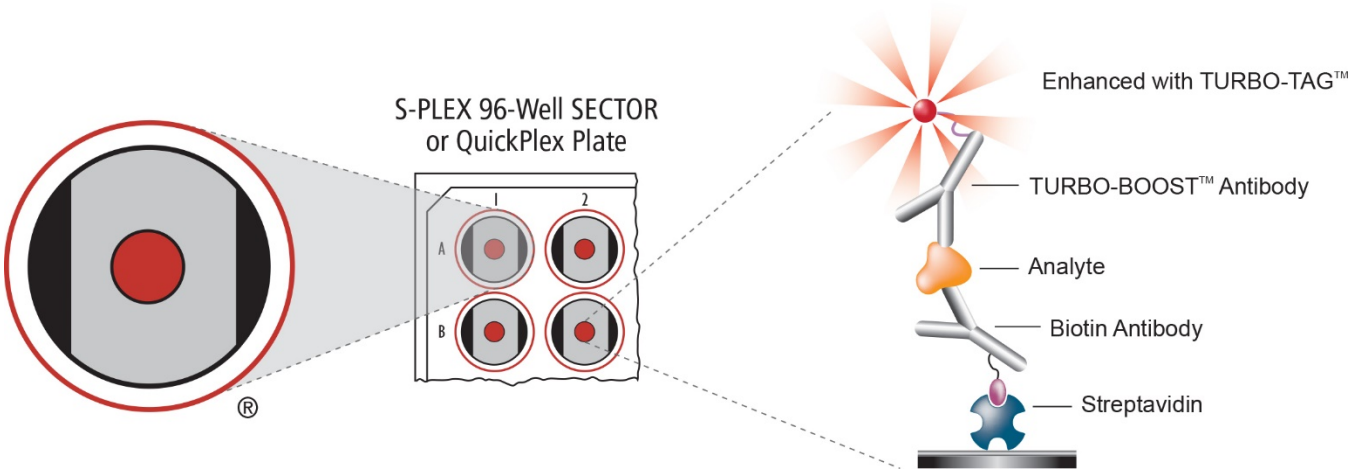

Figure 1. S-PLEX singleplex assay on an S-PLEX 96-well SECTOR or QuickPlex plate.

Performing an S-PLEX assay is similar to other MSD assays. The protocol is simple, robust, and uses common laboratory techniques. A graphical representation of the protocol is shown in Figure 2. The steps are outlined below:

ASSEMBLE

- ❑ Prepare coating solution containing biotin-conjugated capture antibody and S-PLEX Coating Reagent C1.
- ❑ Coat S-PLEX Plate.
- ❑ Add samples and calibrators.
- ❑ Add TURBO-BOOST detection antibody.

ENHANCE

- ❑ Add S-PLEX enhance solution.
- ❑ Add S-PLEX detection solution. This detection solution includes the TURBO-TAG label that is required for the electrochemiluminescent signal. During this step, TURBO-TAG binds to the enhanced TURBO-BOOST. TURBO-BOOST or TURBO-TAG alone will not generate any signal.

READ

- ❑ Add MSD Read Buffer and read on an MSD instrument.

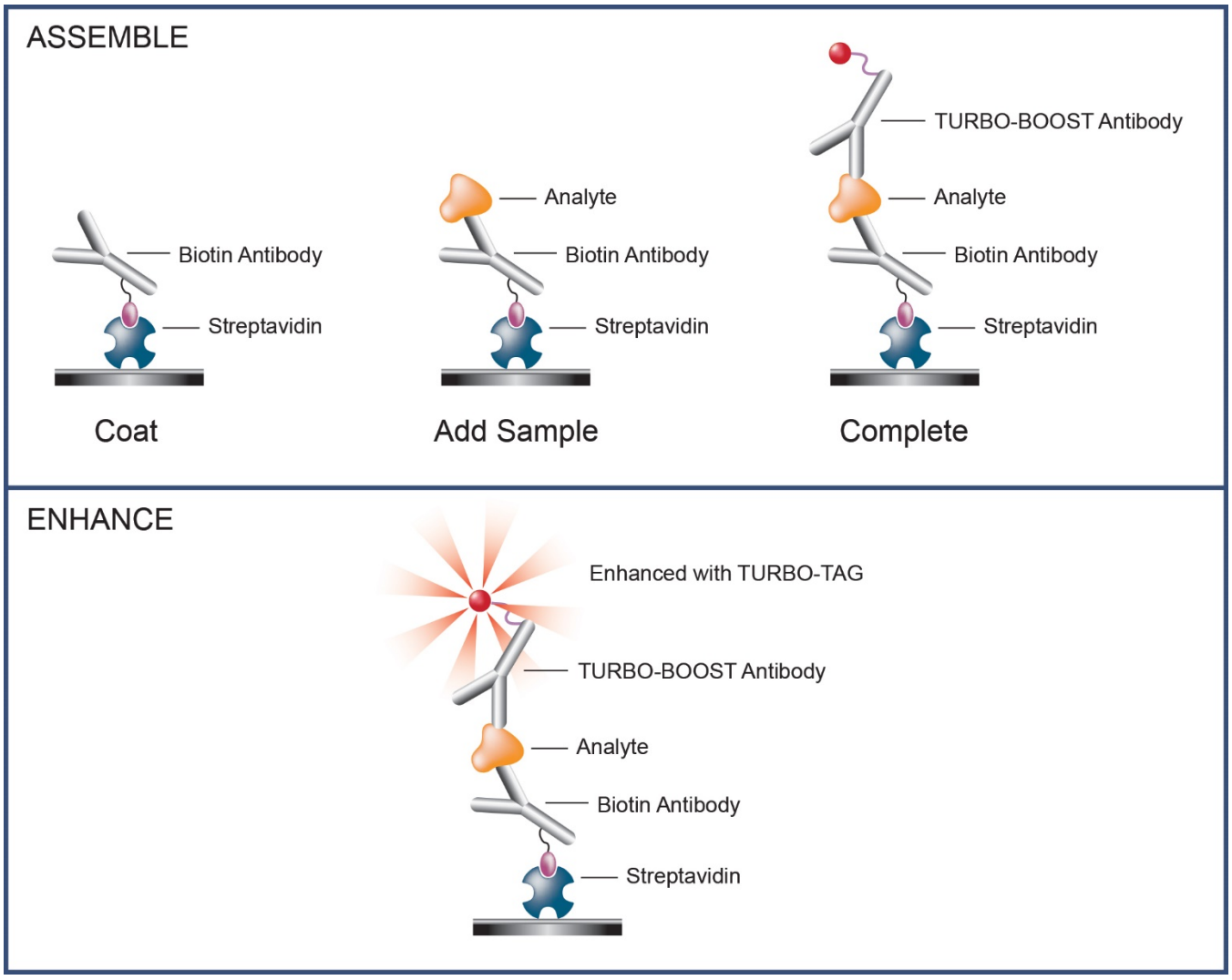

Figure 2. S-PLEX assay format on an S-PLEX 96-well SECTOR or QuickPlex plate.

Kit Components

S-PLEX assay kits are available as Singleplex assays in 1, 5, and 25 plates size. S-PLEX assay kits include kit lot-specific (Table 1) and non-kit lot-specific reagents (Table 2, Table 3). Assay kits are available in two plate formats compatible with either SECTOR or QuickPlex instruments (Table 3).

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

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

Kit Lot-Specific Reagents and Components

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

Reagent	Cap color	Storage	Catalog #	Size	Quantity Supplied			Description
					1 Plate	5 Plates	25 Plates	
Biotin Human IFN-γ Antibody	○	2–8 °C	C21X9-2	170 µL	1	-	-	Assay-specific biotinylated capture antibody
			C21X9-3	850 µL	-	1	5	
TURBO-BOOST Human IFN-γ Antibody	●	2–8 °C	D21X9-2	45 µL	1	-	-	TURBO-BOOST conjugated detection antibody
			D21X9-3	225 µL	-	1	5	
Human IFN-γ Calibrator	-	2–8 °C	C01X9-2	1vial	1 vial	5 vials	25 vials	Contains analyte of known concentration. Used for creating the standard curve for each assay
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 non-specific 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
Diluent 58		≤-10 °C	R50CA-1	10 mL	1 bottle	-	-	Assay diluent for samples and Calibrator
			R50CA-2	50 mL	-	1 bottle	5 bottles	

All reagents listed above are kit lot-specific. Lot-specific information for each assay can be found in the certificate of analysis (COA).

RT = room temperature.

- = not applicable.

Non-Kit Lot-Specific Reagents and Components

Table 2. Non-kit lot-specific reagents and components that are supplied with the S-PLEX Human IFN-γ Kit

Reagent	Storage	Catalog #	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	
MSD GOLD™ Read Buffer B	RT	R60AM-1	18 mL	1 bottle	-	-	Buffer to catalyze the electrochemiluminescence reaction
		R60AM-2	90 mL	-	1 bottle	5 bottles	

RT = room temperature.

- = not applicable.

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

Reagent	Storage	Catalog #	Quantity Supplied			Instrument Compatibility	Description
			1 Plate	5 Plates	25 Plates		
S-PLEX 96-Well 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 QuickPlex Plate	2–8 °C	L4BNA-1	1 plate	5 plates	25 plates	MESO QuickPlex Q 60MM	

Additional Materials and Equipment

Materials

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

Equipment

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

Safety

Use safe laboratory practices: wear gloves, safety glasses, and lab coats when handling assay components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional product-specific safety information is available in the applicable safety data sheet(s), which can be obtained from MSD Customer Service or at www.mesoscale.com.

Best Practices

- Mixing and substituting reagents from different sources or different kit lots is not recommended. Lot information is provided in the lot-specific COA.
- Bring frozen diluents to room temperature in a 22–25 °C water bath prior to use. If a controlled water bath is not available, thaw at room temperature. Ensure that diluents are fully thawed and equilibrated to room temperature before use. Mix well after thawing and before use.
- To avoid cross-contamination between vials, open vials for one protocol step at a time (vial caps are color-coded). Use filtered pipette tips and use a fresh pipette tip for each reagent addition.
- Prepare Calibrators and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by vortexing after each dilution.
- Avoid bubbles in wells during all pipetting steps as they may lead to variable results. Bubbles introduced when adding read buffer may interfere with signal detection.
- Use reverse pipetting when necessary to avoid the introduction of bubbles. For empty wells, pipette gently to the bottom corner. Do not touch the pipette tip on the bottom of the wells when pipetting into the MSD plate.
- Plate shaking should be vigorous, with a rotary motion between 500 –1,000 rpm. Binding reactions may reach equilibrium sooner if shaken in the middle of this range (~700 rpm) or above.
- Use a new adhesive plate seal for all incubation steps.
- When using an automated plate washer, use individual wash cycles, and rotate the plate 180 degrees between wash steps to improve assay precision and reduce potential assay issues due to washing.
- When performing manual plate washing using a multi-channel pipette, plates should be washed using at least 150 µL of wash buffer per well.
- Gently tap the plate on a paper towel to remove residual fluid after washing.
- Avoid excessive drying of the plate during washing steps. Add solutions to the plate immediately after washing.
- Remove the plate seal prior to reading the plate.
- Make sure that the Read Buffer is at room temperature when adding to the plate.
- Do not shake the plate after adding Read Buffer.
- To improve inter-plate precision, keep time intervals consistent between adding Read Buffer and reading the plate. Unless otherwise directed, read the plate as soon as possible after adding Read Buffer.
- If the sample results are above the top of the calibration curve, dilute the samples, and repeat the assay.
- If the sample requires higher dilutions, Diluent 100 may be used in place of assay diluent.
- When running a partial plate, seal the unused sectors to avoid contaminating unused wells. Remove all seals before reading. Partially used plates may be stored up to 30 days at 2–8 °C in the original foil pouch with desiccant. You may adjust volumes proportionally when preparing reagents.
- 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. During the detection incubation step, plates do not need to be shielded from light except for direct sunlight.
- When washing S-PLEX assays, best results are obtained by using a low dispense flow rate and by positioning dispenser tips at the outer edge of the well (e.g., horizontal dispense offset towards the left side of the well). This is most important after the detection solution incubation step. See **Appendix A** for more information on plate washing recommendations.

Recommended Protocol

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

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

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

STEP 1: ASSEMBLE

Prepare Coating Solution

Biotinylated capture antibody is provided as a 40X stock solution and S-PLEX Coating Reagent C1 as a 200X stock solution. Thaw frozen vials and bring all reagents to room temperature. Vortex each vial to mix and spin down briefly before use.

- ❑ Prepare the coating solution immediately prior to use by combining the following reagents. Vortex briefly to mix.
  - ❑ 5,820 µL Diluent 100
  - ❑ 150 µL of Biotin Human IFN-γ Antibody ○
  - ❑ 30 µL of 200X S-PLEX Coating Reagent C1 ●

Notes:

- **CRITICAL:** Failure to add S-PLEX Coating Reagent C1 in the coating solution will drastically reduce the assay signal.
- The unused S-PLEX Coating Reagent C1 should be frozen immediately after use. The reagent is stable through 5 freeze-thaw cycles.

➢ Coat the Plate

- ❑ Wash the uncoated plates 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T (PBS plus 0.05% Tween-20). Pre-washing the plate has shown to increase signals and improve sensitivity in many assays.
- ❑ Add 50 µL of coating solution to each well. Tap the plate gently on all sides. Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at room temperature for 1 hour or overnight at 2–8 °C. Shaking is not required for overnight coating incubation.

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

Prepare Blocking Solution

Blocking solution is the assay diluent supplemented with Blocker S1, and is designed to reduce non-specific binding in the sample matrix. Blocker S1 is provided as a 100X stock solution. Thaw frozen vials and bring all reagents to room temperature. Vortex each vial to mix and spin down briefly before use.

- ❑ Prepare the blocking solution by combining the following reagents. Vortex briefly to mix.
  - ❑ 3,465 µL of Diluent 58
  - ❑ 35 µL of 100X Blocker S1 ◯

Notes:

- 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.
- The blocking solution should be added to the plate before sample addition.

Prepare Calibrator Dilutions

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

Prepare the highest calibrator concentration (Standard 1):

- ❑ Reconstitute lyophilized Human IFN-γ Calibrator by adding 1,000 µL of Diluent 58 to the vial. Invert at least 3 times (do not vortex). Let the reconstituted solution equilibrate at room temperature for 15–30 minutes, and then vortex briefly using short pulses.

Note: Reconstituted calibrator is not stable when stored at 2–8 °C; however, it may be stored in aliquots at ≤-70 °C and is stable for one freeze-thaw cycle. 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 remaining standards plus a zero standard for up to 4 replicates (Figure 3):

- ❑ Prepare Standard 2 by adding 50 µL of Standard 1 to 150 µL of Diluent 58. Mix by vortexing.
- ❑ Repeat 4-fold serial dilutions five additional times to generate Standards 3–7. Mix by vortexing between each serial dilution.
- ❑ Use Diluent 58 as Standard 8 (zero standard).

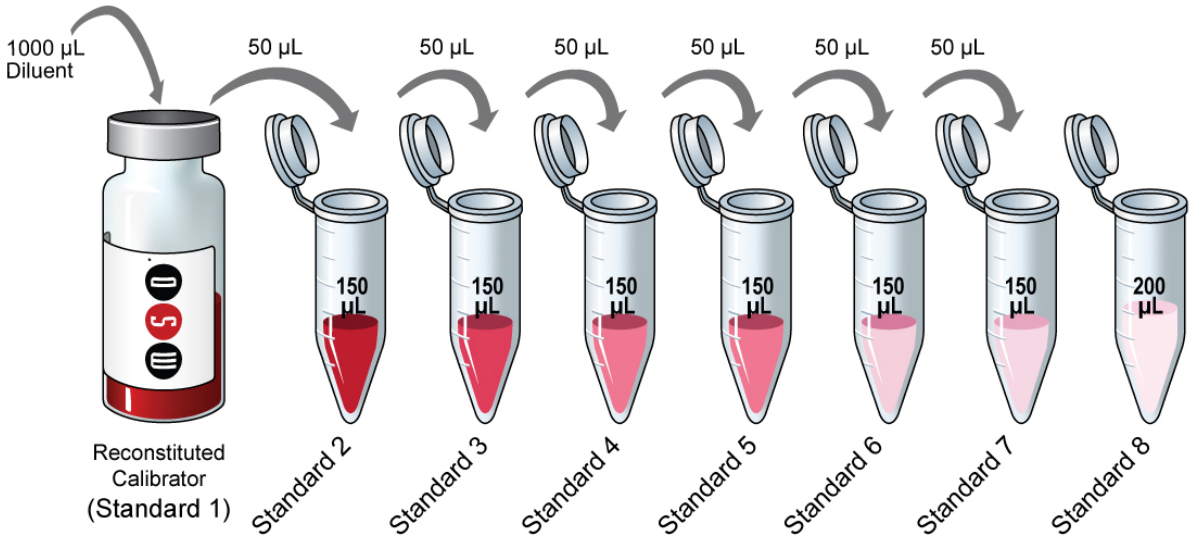

Figure 3. Dilution schema for preparation of calibrator standards.

Sample Collection and Handling

Below are general guidelines for sample collection, storage, and handling. 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 x g prior to using or freezing. Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 20 minutes at 2,000 x g within 30 minutes of collection. Use immediately or freeze.
- **CSF**. MSD recommends reviewing current literature and protocols for collection and handling of CSF samples or use 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 x g for 3 minutes to remove particulates prior to sample preparation. Hold on wet ice or at 2–8 °C until used in the assay.

Dilute Samples

Serum, plasma, and CSF samples do not require dilution for measuring IFN-γ. The assay requires 25 µL/well of sample. You may conserve sample by using a higher dilution. The dilution factor for other sample types will need to be optimized. Additional diluent can be purchased at www.mesoscale.com.

➢ Add Calibrators and Sample

- ❑ After coating incubation completion, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T (PBS plus 0.05% Tween-20).
- ❑ Add 25 µL of blocking solution to each well. Tap the plate gently on all sides.
- ❑ Add 25 µL of calibrator or sample to each well.
- ❑ Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at room temperature for 1.5 hours.

Prepare TURBO-BOOST Antibody Solution

TURBO-BOOST detection antibody is provided as a 200X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately prior to use. Bring all reagents to room temperature. Spin down the vial before use.

- ❑ Prepare the TURBO-BOOST antibody solution by combining the following reagents. Vortex briefly to mix.
  - ❑ 5,970 µL of Diluent 59
  - ❑ 30 µL of TURBO-BOOST Human IFN-γ Antibody

➢ Add TURBO-BOOST Antibody Solution

- ❑ After calibrator and sample incubation, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T (PBS plus 0.05% Tween-20).
- ❑ Add 50 µL of TURBO-BOOST antibody solution to each well.
- ❑ Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at room temperature for 1 hour.

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

STEP 2: ENHANCE

Prepare Enhance Solution

Prepare the enhance solution up to 30 minutes prior to use. Thaw frozen vials and bring all reagents to room temperature. Vortex each vial to mix and spin down briefly before use.

- ❑ Prepare enhance solution by combining the following reagents. Vortex briefly to mix.
  - ❑ 2,970 µL Molecular Biology Grade water
  - ❑ 1,500 µL of 4X S-PLEX Enhance E1
  - ❑ 1,500 µL of 4X S-PLEX Enhance E2
  - ❑ 30 µL of 200X S-PLEX Enhance E3

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

- ➢ Add Enhance Solution
  - ❑ After TURBO-BOOST antibody incubation, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T (PBS plus 0.05% Tween-20).
  - ❑ Add 50 µL of enhance solution to each well.
  - ❑ Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at room temperature for **30 minutes**.

Note: While the enhance solution is incubating, thaw 1 vial each of S-PLEX D1 and D2 reagents at room temperature.

Prepare TURBO-TAG Detection Solution

Prepare the TURBO-TAG detection solution up to 30 minutes prior to use. Thaw frozen vials and bring all reagents to room temperature. Vortex each vial to mix and spin down briefly before use.

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

Notes:

- **CRITICAL:** Avoid prolonged exposure of the S-PLEX Detect D1 reagent and detection solution to light.
- S-PLEX Detect D2 solution is viscous. Pipette slowly to avoid bubble formation in the tip and to ensure accurate pipetting volume.
- **CRITICAL:** The TURBO-TAG detection incubation (next-step) requires incubation at 27 °C. Upon completion of the enhance solution incubation, prepare a shaker at 27 °C.

- ➢ Add TURBO-TAG Detection Solution
  - ❑ After enhance solution incubation, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T (PBS plus 0.05% Tween-20).
  - ❑ Add 50 µL of TURBO-TAG detection solution to each well.
  - ❑ Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at **27 °C** for 1 hour.

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

STEP 3: READ

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

- **Add Read Buffer**
  - ❑ After TURBO-TAG detection incubation, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T (PBS plus 0.05% Tween-20) using a gentle wash step.

Note: **CRITICAL:** For this final wash step, best results are obtained by using a low dispense flow rate and by positioning dispense tips at the outer edge of the well (e.g. horizontal dispense offset towards the left side of the wall). See **Appendix A** for more information on plate washing recommendations if using an automated plate washer.

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

Note: **CRITICAL:** Refer to the plate-instrument compatibility table (Table 3) to ensure correct plate is read on the compatible instrument. SECTOR plates are compatible with SECTOR and QuickPlex SQ instruments. QuickPlex plates are **ONLY** compatible with the QuickPlex Q 60MM instrument.

Assay Performance

A representative data set for the S-PLEX Human IFN-γ assay is presented below and is also available at www.mesoscale.com. The data represent the performance of the assay tested in singleplex format. The data were generated during the development of the assay and do not represent the product specifications. Under your experimental conditions, the assay may perform differently than the representative data shown.

Representative Calibrator Curve and Sensitivity

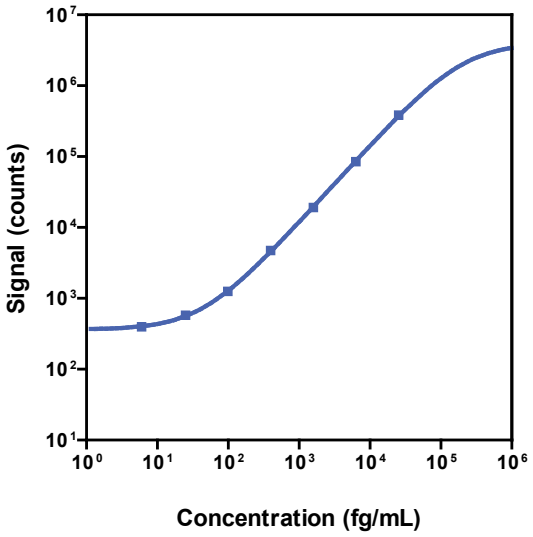

Table 4. LLOD, LLOQ, and ULOQ for the S-PLEX Human IFN-γ Kit

Suggested Sample Dilution	Neat
LLOD (fg/mL)	5.3
LLOQ (fg/mL)	16
ULOQ (fg/mL)	15,000

Figure 4. Typical calibrator curve for the S-PLEX Human IFN-γ 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. The lower limit of detection (LLOD) is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero Standard). The upper limit of quantification (ULOQ) is the highest concentration at which the CV of calculated concentration is <25% and the recovery of each analyte is within 75% to 125% of the known value. The lower limit of quantification (LLOQ) is the lowest concentration at which the CV of calculated concentration is <25% and the recovery of each analyte is within 75% to 125% of the known value. Analyte concentrations were determined from the electrochemiluminescence signals by back-fitting to the calibration curve.

Tested Samples

Normal human serum, EDTA plasma, citrate plasma, heparin plasma, and CSF samples were tested without dilution. Cell culture supernatant samples were tested at multiple dilutions (50–10,000 fold dilutions). Concentrations reported in the table are adjusted for sample dilution.

Table 5. Samples tested in the S-PLEX Human IFN-γ Kit

Statistics	Serum (N = 25)	EDTA Plasma (N = 15)	Citrate Plasma (N = 15)	Heparin Plasma (N = 15)	CSF (N =10)	Cell Culture Supernatant (N = 4)
Median (fg/mL)	360	370	320	390	150	AS
Range (fg/mL)	150–6,100	150–880	130–900	150–1,000	19–AS	85–AS
% Detected	100	100	100	100	100	100

ND = non-detectable (<LLOD)

AS = above Standard 1.

Parallelism/Dilution Linearity

Normal human serum, EDTA plasma, citrate plasma, and heparin plasma samples were tested at different dilutions. Cell culture media 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.

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

Table 6. Analyte percent recovery at various fold dilutions of each sample type

Fold Dilution	Serum		EDTA Plasma		Citrate Plasma		Heparin Plasma		Cell Culture Media	
	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Neat	100	NA	100	NA	100	NA	100	NA	100	NA
2	112	108–117	117	110–133	112	109–116	107	103–111	98	97-98
4	107	97–114	113	109–123	103	98–111	94	88–110	97	95-98
8	118	101–150	119	111–124	107	97–117	101	98–105	97	92-98

NA = not applicable.

Spike Recovery

Normal human serum, EDTA plasma, citrate plasma, heparin plasma, and cell culture media samples were spiked with calibrator at 3 levels. Spiked samples were tested neat. Samples may require additional dilution with assay diluent to reduce matrix effects.

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

Table 7. Spike and recovery measurement of different sample types at three spiked levels

Spike Level	Serum		EDTA Plasma		Citrate Plasma		Heparin Plasma		Cell Culture Media	
	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
High	99	78–111	91	82–95	101	90–111	99	94–103	85	84–85
Mid	99	88–111	82	75–86	92	86–97	91	85–97	85	83–86
Low	97	88–107	85	81–92	91	86–98	95	89–100	87	77–96

Specificity

To assess specificity, the S-PLEX Human IFN-γ assay was tested against a larger panel of human analytes for nonspecific binding (Eotaxin, Eotaxin-3, EPO, FLT3L, G-CSF, GM-CSF, IFN-β, IFN-α2a, IL-10, IL-12/23p40, IL-12p70, IL-13, IL-15, IL-16, IL-16, IL-17A, IL-17A/F, IL-17B, IL-17C, IL-17D, IL-17E/IL25, IL-17F, IL-18, IL-1α, IL-1β, IL-1RA, IL-2, IL-21, IL-22, IL-23, IL-27, IL-29, IL-2RA, IL-3, IL-31, IL-33, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, MIP-3α, TARC, TNF-α, TNF-β, TPO, TSLP, VEGF-A).

Nonspecific binding was less than 0.5%.

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

Assay Components

Calibrators

The assay calibrator uses the following recombinant human protein:

Table 8. Recombinant human proteins used in the calibrator

Calibrator	Expression System
IFN-γ	*E. coli*

Antibodies

Table 9. Antibody source species

Analyte	Source Species		Assay Generation
	MSD Capture Antibody	MSD Detection Antibody	
IFN-γ	Mouse Monoclonal	Mouse Monoclonal	A

References

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

Appendix A: Recommended Plate Washer Parameters

When using an automated plate washer for S-PLEX assays, 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). The only different parameters are the Dispense Rate and Dispense X-Position.

Table 10. Parameters for customized programs on the Biotek 405 LS Microplate Washers

Wash Program Parameters	Typical Wash Program Settings	Recommended S-PLEX 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/sec)	1 (4.1% 1.0 mm/sec)
Aspirate Delay	0500 msecs	0500 msecs
Aspirate X-Position	-35 (1.600 mm)	-35 (1.600 mm)
Aspirate Y-Position	-35 (1.600 mm)	-35 (1.600 mm)
Asp Height	22	22
Secondary Asp?	NO	NO
DISPENSE		
Dispense Rate	05	**02**
Dispense Volume	0300 µL/well	0300 µL/well
Vacuum Delay Vol	0300 µL/well	0300 µL/well
Dispense X-Position	00 (0.000 mm)	**-35 (1.600 mm)**
Dispense Y-Position	00 (0.000 mm)	00 (0.000 mm)
Dispense Height	120 (15.245 mm)	120 (15.245 mm)
OPTS		
PRE		
Wash Pre-dispense?	NO	NO
Bottom Wash?	NO	NO
MIDCYC		
Wash Shake?	NO	NO
Wash Soak?	NO	NO
Home Carrier?	NO	NO
Between Cyc PreDisp?	NO	NO
POST		
Final Aspirate?	YES	YES
Aspirate Type	TOP	TOP
Travel Rate	3	3
Fin Asp Delay	0500 msecs	0500 msecs
Fin Asp X-Position	-35 (1.600 mm)	-35 (1.600 mm)
Fin Asp Y-Position	-35 (1.600 mm)	-35 (1.600 mm)
Fin Asp Height	22	22
Secondary Aspirate?	YES	YES
Fin Asp Sec X-Pos	35 (1.600 mm)	35 (1.600 mm)
Fin Asp Sec Y-Pos	35 (1.600 mm)	35 (1.600 mm)
Fin Asp Sec Height	22	22

Appendix B: Frequently Asked Questions

1. **Can I use a one-step dilution to make the top standard instead of using a 2-step or 3-step dilution?**

   You can perform dilutions with volumes other than defined in the protocol. We recommend not to pipette volumes less than 10 µL. If using volumes less than 10 µL, ensure that pipettes are appropriately calibrated to accurately dispense small volumes. Make sure you prepare ~150 µL of Standard 1 after performing intermediate dilutions. However, for consistent and reproducible performance, we recommend following the instructions as outlined in the protocol.

2. **Can I extend capture, sample, and detection antibody incubation time?**

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

3. **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.

4. **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.

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

   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.

6. **When should I thaw my reagents?**

   - **Enhance Solution**: Start thawing E1, E2, and E3 at room temperature 30 minutes after the start of TURBO-BOOST antibody incubation.
   - **TURBO-TAG Detection Solution**: Start thawing D1 and D2 at room temperature, right after the start of the incubation of enhance solution.

7. **Which reagents are recommended to be stored on ice, what stocks should be stored in the dark?**

   If either E3 or D2 needs to be used repeatedly, we recommend storing them on ice (they thaw completely on ice rapidly). D1 should be treated similarly to SULFO-TAG conjugated antibodies, and prolonged light exposures should be avoided.

8. 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.

9. 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.

10. What volume of wash buffer is needed during plate washing?

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

Summary Protocol

STEP 1: ASSEMBLE

- **Coat Plate with Biotin Antibody**
  - ❑ Pre-wash plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
  - ❑ Add 50 µL of coating solution containing biotinylated capture antibody and Coating Reagent C1to each well. Tap the plate gently on all sides. Seal plate with an adhesive plate seal.
  - ❑ Incubate at room temperature with shaking (700 rpm) for 1 hour, or overnight without shaking at 2–8 °C.
- **Add Samples and Calibrators**
  - ❑ Wash plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
  - ❑ Add 25 µL of blocking solution to each well. Tap the plate gently on all sides.
  - ❑ Add 25 µL of calibrator or sample to each well. Seal plate with an adhesive plate seal.
  - ❑ Incubate at room temperature with shaking (700 rpm) for 1.5 hours.
- **Add TURBO-BOOST Antibody Solution**
  - ❑ Wash plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
  - ❑ Add 50 µL of TURBO-BOOST antibody solution to each well. Seal plate with an adhesive plate seal.
  - ❑ Incubate at room temperature with shaking (700 rpm) for 1 hour.

STEP 2: ENHANCE

- **Add Enhance Solution**
  - ❑ Wash plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
  - ❑ Add 50 µL of enhance solution to each well. Seal plate with an adhesive plate seal.
  - ❑ Incubate at room temperature with shaking (700 rpm) for 30 minutes.
- **Add TURBO-TAG Detection Solution**
  - ❑ Wash plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
  - ❑ Add 50 µL of TURBO-TAG detection solution to each well. Seal plate with an adhesive plate seal.
  - ❑ Incubate at 27 °C in a temperature controlled shaker with shaking (700 rpm) for 1 hour.

STEP 3: READ

- **Add Read Buffer**
  - ❑ Wash plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T using washer program with low dispense speed. See **Appendix A** for more details.
  - ❑ Add 150 µL of MSD GOLD Read Buffer B to each well. Read the plate on an MSD instrument. Incubation in MSD GOLD Read Buffer B is not required before reading the plate.

Catalog Numbers

Table 11. Catalog numbers associated with the S-PLEX Human IFN-γ 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-γ	K151X9S-1	K151X9S-2	K151X9S-4	K151X9S-21	K151X9S-22	K151X9S-24

Plate Diagram

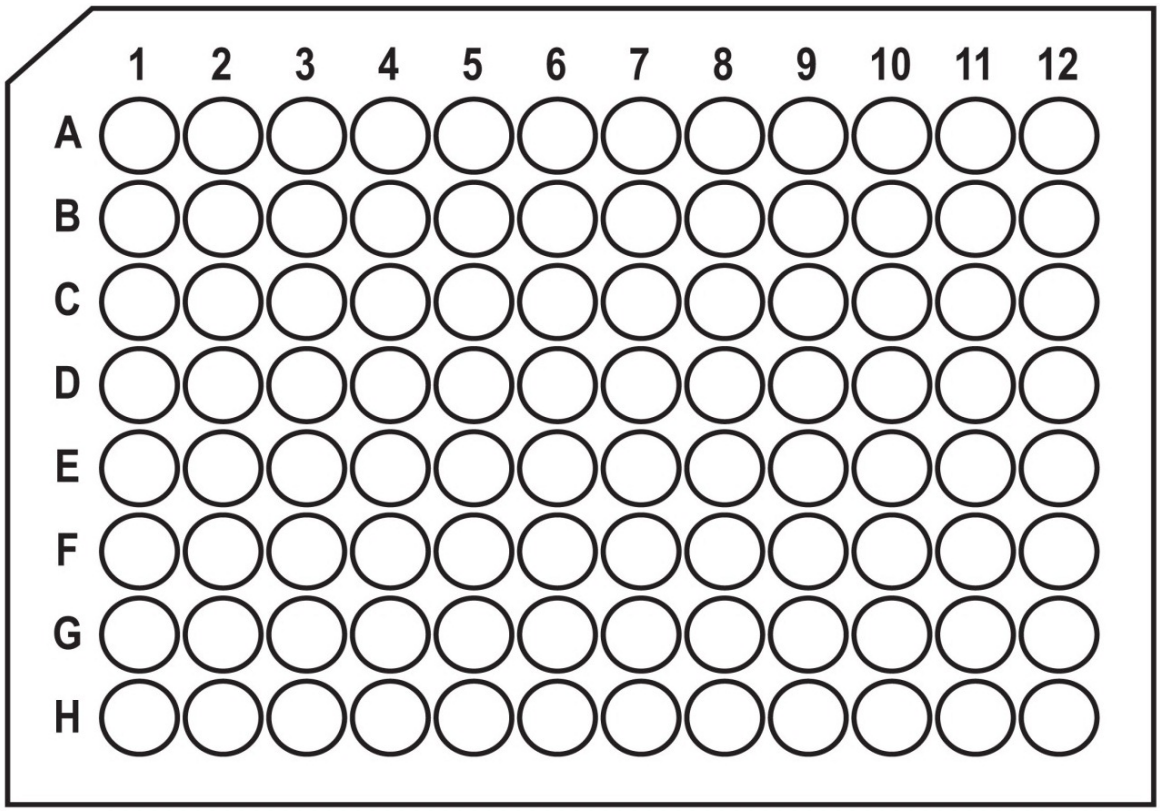

Figure 5. Plate Diagram.