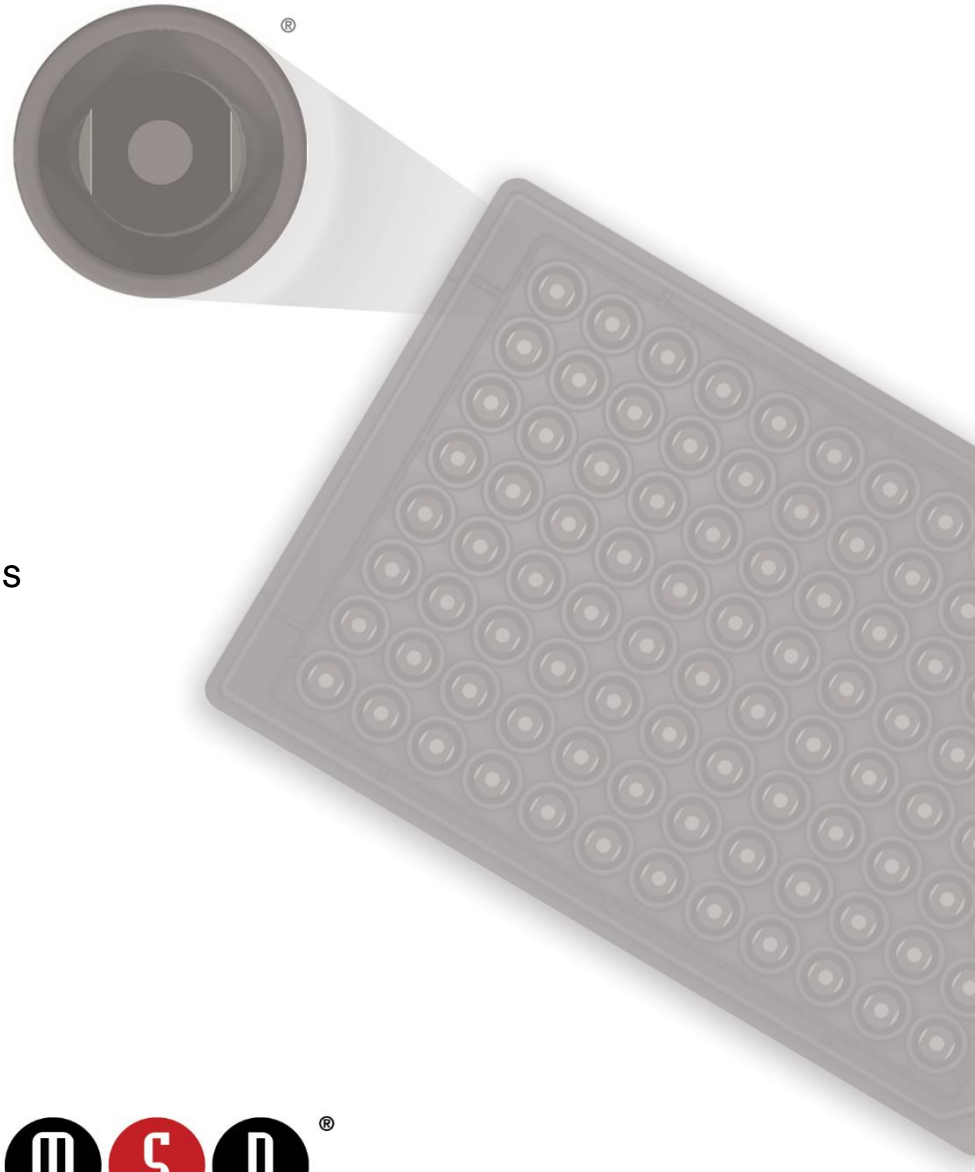
MSD® S-PLEX Platform

SARS-CoV-2 N Kit

S-PLEX® TrueSensitivity®



	S-PLEX®
SARS-CoV-2 N Kit	K150ADHS

MSD S-PLEX Platform

S-PLEX SARS-CoV-2 N Kit

For the detection of Severe Acute Respiratory Syndrome Coronavirus 2 Nucleocapsid Protein (SARS-CoV-2 N) in human serum, EDTA plasma, saliva, and nasopharyngeal swab (upper respiratory) samples.

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 MESO SCALE DISCOVERY® S-PLEX SARS-CoV-2 N Kit is an ultrasensitive immunoassay that measures Severe Acute Respiratory Syndrome Coronavirus 2 Nucleocapsid Protein (SARS-CoV-2 N) in multiple human sample types.

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 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, reduce required sample volumes, 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 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.

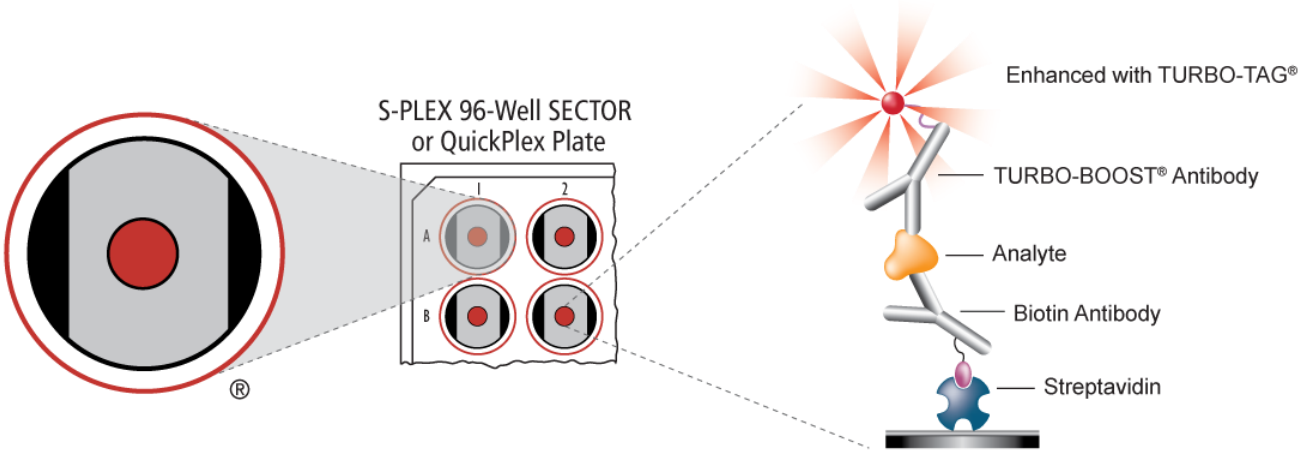

Figure 1. S-PLEX Singleplex Assay on an S-PLEX Plate.

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
- ❑ Coat S-PLEX Plate.
- ❑ Add samples and calibrators.
- ❑ Add TURBO-BOOST detection antibody.

ENHANCE

- ❑ Add S-PLEX enhance solution.
- ❑ Add S-PLEX detection solution.

READ

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

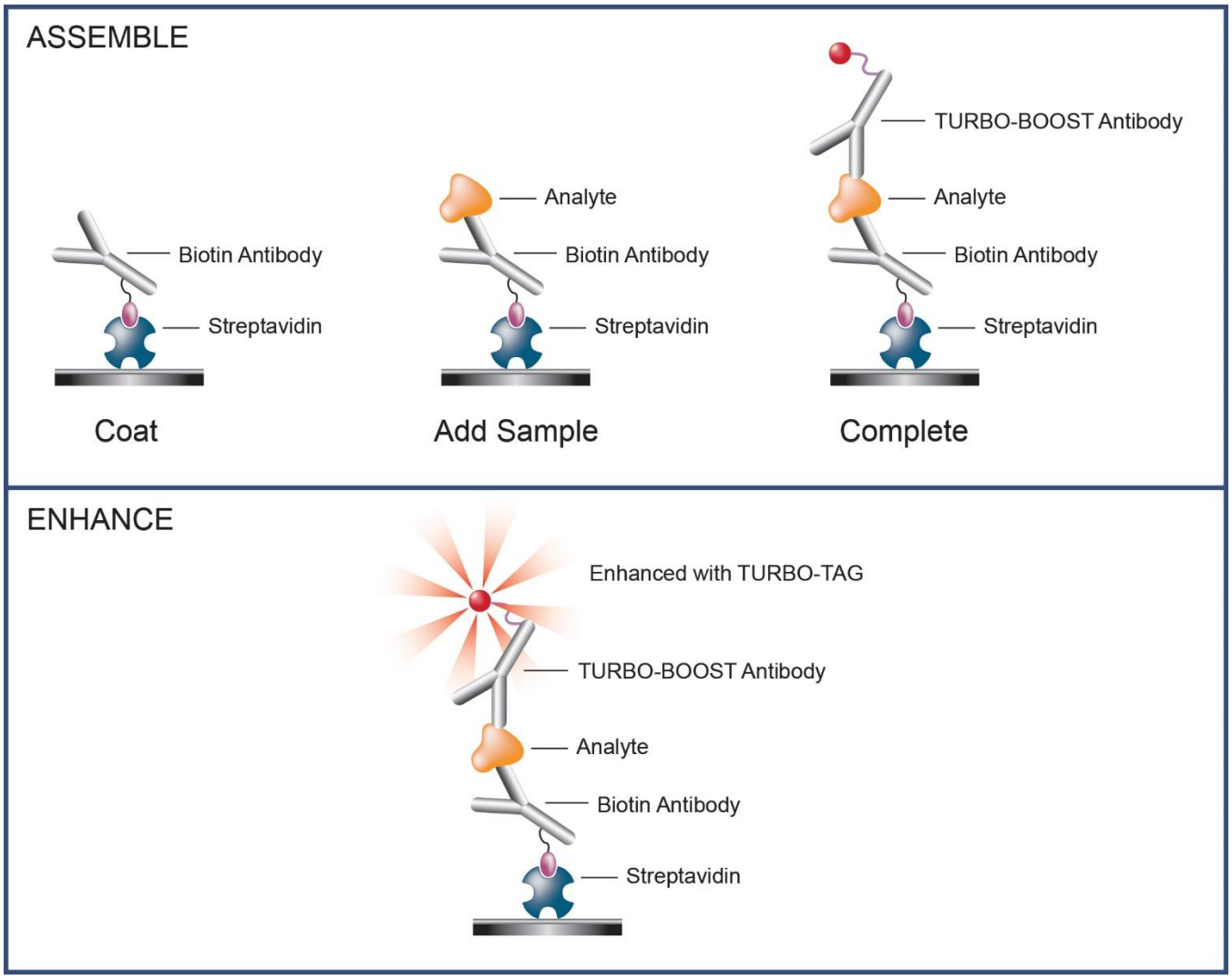

Figure 2. S-PLEX Assay Format.

Kit Components

S-PLEX assay kits are available as Singleplex assays in 1, 5, and 25 plates size.

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 SARS-CoV-2 N Kit

Reagent	Cap color	Storage	Catalog #	Size	Quantity Supplied			Description
					1 Plate	5 Plates	25 Plates	
Biotin SARS-CoV-2 N Antibody	○	2–8 °C	C20AUH-2	170 µL	1	-	-	Assay-specific biotinylated capture antibody
			C20AUH-3	850 µL	-	1	5	
TURBO-BOOST SARS-CoV-2 N Antibody	●	2–8 °C	D20AUH-2	45 µL	1	-	-	TURBO-BOOST conjugated detection antibody
			D20AUH-3	225 µL	-	1	5	
SARS-CoV-2 N Calibrator	-	≤-70 °C	C00ADH-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 61		≤-10 °C	R50CD-1	8 mL	1 bottle	-	-	Assay diluent for samples and Calibrator
			R50CD-2	40 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 SARS-CoV-2 N 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., BioSan PST-60HL-4)
- ❑ 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, E1, E2, and D1 reagents 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, E1, E2, and D1 reagents are fully thawed and equilibrated to room temperature before use. Mix well after thawing and before use.
- Thaw frozen vials of E3 and D2 reagents on ice until needed. Ensure that E3 and D2 reagents are fully thawed before use. Mix well after thawing and before use.
- To avoid cross-contamination between vials, open vials for 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 washing S-PLEX Assays, the best results are obtained by using a low dispense flow rate and by positioning dispenser tips at the outer edge of the well (e.g., horizontal dispense offset towards the left side of the well). This is most important after the Detection Solution incubation step. See **Appendix A** for more information on plate washing recommendations.
- When performing manual plate washing using a multi-channel pipette, plates should be washed using at least 150 µL of wash buffer per well. Excess residual volume after washing should be removed by gently tapping the plate on a paper towel.
- Do not allow plates to dry after washing steps. Solutions associated with the next assay step should be added to the plate immediately after washing.
- Remove the plate seal 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 interplate precision, keep time intervals consistent between adding read buffer and reading the plate. Unless otherwise directed, read the plate as soon as possible after adding read buffer.
- If the sample requires higher dilutions, Diluent 100 may be used in place of assay diluent.
- Avoid prolonged exposure of the S-PLEX Detect D1 reagent and detection solutions to light. Keep stocks of S-PLEX Detect D1 reagent in the dark.
- Intense light sources can affect assay performance. Plates should be protected from direct light during the plate shaking steps for optimal results.

Recommended Protocol

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

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

CAUTION: Concentrations of SARS-CoV-2 protein in some clinical sample types have a wide range and can be orders of magnitude above the upper end of the assay range. To avoid inaccurate results due to cross-contamination of samples, it is very important to change pipette tips between each set of sample replicates at both the sample addition and TURBO-BOOST reagent addition steps.

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 SARS-CoV-2 N Antibody ○
  - ❑ 30 µL of 200X S-PLEX Coating Reagent C1 ●

Note:

- 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 samples.

Prepare Blocking Solution

Blocker S1 is provided as a 100X stock solution. Thaw the frozen vial 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 61
  - ❑ 35 µL of 100X Blocker S1 ◉

Note:

- 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 at a concentration that is 20-fold higher than the recommended highest standard. We recommend a 7-point calibration curve with 4-fold serial dilution steps and a zero calibrator blank. Thaw the stock calibrator and keep on ice, then add to Blocking Solution at room temperature to make the calibration curve solutions.

Note: Discard any unused, diluted calibrator solutions. For the lot-specific concentration of the calibrator, refer to the COA supplied with the kit. You can also find a copy of the COA at www.mesoscale.com. To prepare seven standard solutions plus a zero calibrator for up to 4 replicates, see Figure 3.

- ❑ Prepare Standard 1 by adding 15 µL of stock calibrator to 285 µL of Blocking Solution. Mix by vortexing.
- ❑ Prepare the next standard solution by transferring 50 µL of Standard 1 to 150 µL of Blocking Solution. Mix by vortexing. Repeat 4-fold serial dilution 5 times to generate Standards 3-7. Mix by vortexing between each serial dilution.
- ❑ Use Blocking Solution as Standard 8 (zero standard).

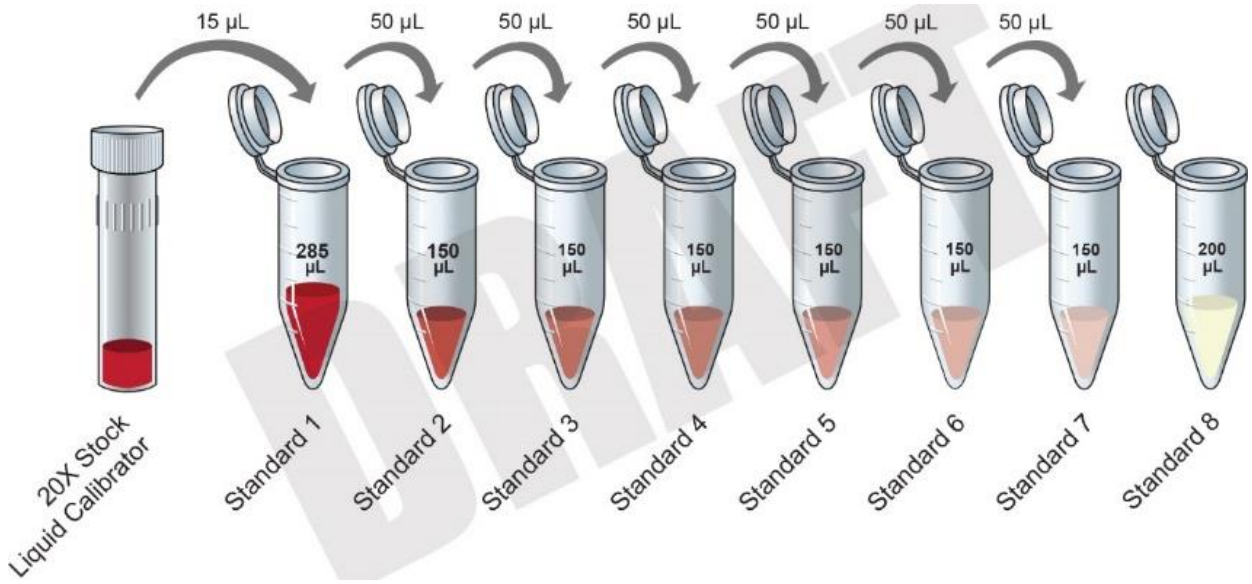

Figure 3. Dilution Schema for Preparation of Calibrator Standards.

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; then centrifuge for 20 minutes at 2,000 x g prior to using or freezing. If no particulates are visible, you may not need to centrifuge. 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.
- Respiratory Swabs. Swab samples should be collected in a non-denaturing transport media such as viral transport medium. Avoid the use of extraction buffers that include protein denaturants that could interfere with antibody recognition of the analyte. Most viral transport media (e.g., UTM) should provide acceptable performance. Other buffers may need to be evaluated for potential interference.
- Saliva. Saliva samples may be tested undiluted, however, if saliva samples tend to be non-homogenous in nature consider diluting 1:4 in Blocking Solution and centrifuging to remove particulates prior to testing.
- 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

Samples were tested neat for measuring SARS-CoV-2 N. You may conserve sample by using a higher dilution. We recommend running at least two replicates per sample. The dilution factor for sample types will need to be optimized. Additional diluent can be purchased at www.mesoscale.com.

Add Calibrators and Sample

- ❑ After coating incubation is complete, 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.

Note: Perform this incubation step between 22 °C and 27 °C.

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 SARS-CoV-2 N 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.

Notes:

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

Notes:

- CRITICAL: Incubation temperatures below 22 °C for this step can negatively affect assay signals and sensitivity. For best results, perform this incubation step between 22 °C and 27 °C.
- While the enhance solution is incubating, thaw 1 vial each of S-PLEX Detect D1 at room temperature and Detect D2 on ice.
- CRITICAL: The TURBO-TAG detection incubation (next step) requires incubation at 27 °C. Upon completion of the enhance solution incubation, prepare a shaker at 27 °C. If you do not have access to a temperature-controlled shaker, a plate shaker can be placed inside an incubator maintaining 27 °C.

Prepare TURBO-TAG Detection Solution

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

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 the TURBO-TAG detection incubation be performed at 27 °C. If you do not have access to a temperature-controlled shaker, a plate shaker can be placed inside an incubator maintaining 27 °C.

STEP 3: READ

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

Notes:

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

Add Read Buffer

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

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

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

Assay Performance

A representative data set for the S-PLEX SARS-CoV-2 N assay is presented below and is also available at www.mesoscale.com. The data represent 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

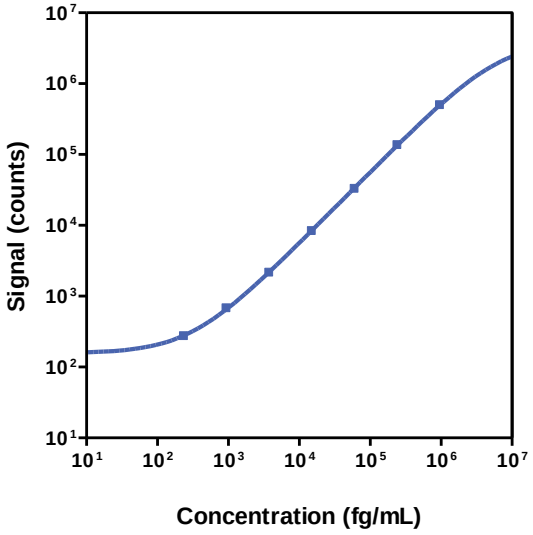

Figure 4. Typical Calibrator Curves for the S-PLEX SARS-CoV-2 N Kit.

Table 4. LLOD, LLOQ, and ULOQ for the S-PLEX SARS-CoV-2 N Kit

Dilution from Stock Liquid Calibrator to Standard 1 (top of curve)	20X
Tested Sample Dilution	Neat
LLOD (fg/mL)	62
LLOQ (fg/mL)	172
ULOQ (fg/mL)	650,000

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 upper limit of quantification (ULOQ) is the highest concentration at which the CV of calculated concentration is <30% and the recovery of each analyte is within 70% to 130% 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.

Tested Samples

Commercially sourced normal human serum, EDTA plasma, and saliva samples were tested without dilution. COVID-19 positive and negative human nasopharyngeal swab (upper respiratory) samples in transport medium were also tested without dilution.

Table 5. Human samples tested in the S-PLEX SARS-CoV-2 N Kit

			Sample Type				
Species	Statistics	Fold Dilution	Nasopharyngeal Swab (Positive) (N = 12)	Nasopharyngeal Swab (Negative) (N = 6)	Saliva (N = 6)	Serum (N = 6)	EDTA Plasma (N = 6)
Human	Median (fg/mL)	Neat	253,000	83	91	85	ND
	Range (fg/mL)		260–AS	ND–83	ND–92	ND–85	ND
	% Detected		100	17	33	17	0

AS = above Standard 1; ND = not detectable (<LLOD).

Dilution Linearity

Commercially sourced normal human serum, EDTA plasma, saliva, and COVID-19 negative human nasopharyngeal swab (upper respiratory) 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 blocking solution to reduce matrix effects.

$$\%\, recovery = \frac{measured\ concentration}{expected\ concentration} X100$$

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

		Nasopharyngeal Swab		Saliva		Serum		EDTA Plasma	
Species	Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Human	Neat	100	NA	100	NA	100	NA	100	NA
	2	123	85–161	90	76–105	127	117–148	134	123–156
	4	136	96–201	83	66–92	140	120–178	153	132–200
	8	144	100–204	81	69–98	141	118–198	179	133–281

NA = not applicable.

Spike Recovery

Commercially sourced normal human serum, EDTA plasma, saliva, and COVID-19 negative human nasopharyngeal swab (upper respiratory) samples were spiked with calibrator at 3 levels. Spiked samples were tested neat. Samples may require additional dilution with blocking solution 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

Species	Spike Level	Nasopharyngeal Swab		Saliva		Serum		EDTA Plasma	
		Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Human	High	101	71–169	167	134–207	77	41–90	76	53–91
	Mid	99	64–151	157	117–203	79	48–95	79	53–88
	Low	95	63–131	155	114–209	77	49–91	79	55–87

Assay Components

Calibrators

The assay calibrator uses the following recombinant human protein:

Table 8. Recombinant Proteins Used in the Calibrator

Calibrator	Expression System
SARS-CoV-2 N	*Human cell line*

Antibodies

Table 9. Antibody Source Species

Analyte	Source Species		Assay Generation
	MSD Capture Antibody	MSD Detection Antibody	
SARS-CoV-2 N	Monoclonal	Monoclonal	B

References

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

Appendix A: Recommended Plate Washer Parameters

When using an automated plate washer for S-PLEX assays, 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 1-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 ºC 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 C1 to each well. Tap the plate gently on all sides. Seal plate with an adhesive plate seal.
  - ❑ Incubate at room temperature with shaking (700 rpm) for 1 hour, 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 chamber 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 SARS-CoV-2 N 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 SARS-CoV-2 N	K150ADHS-1	K150ADHS-2	K150ADHS-4	K150ADHS-21	K150ADHS-22	K150ADHS-24

Plate Diagram

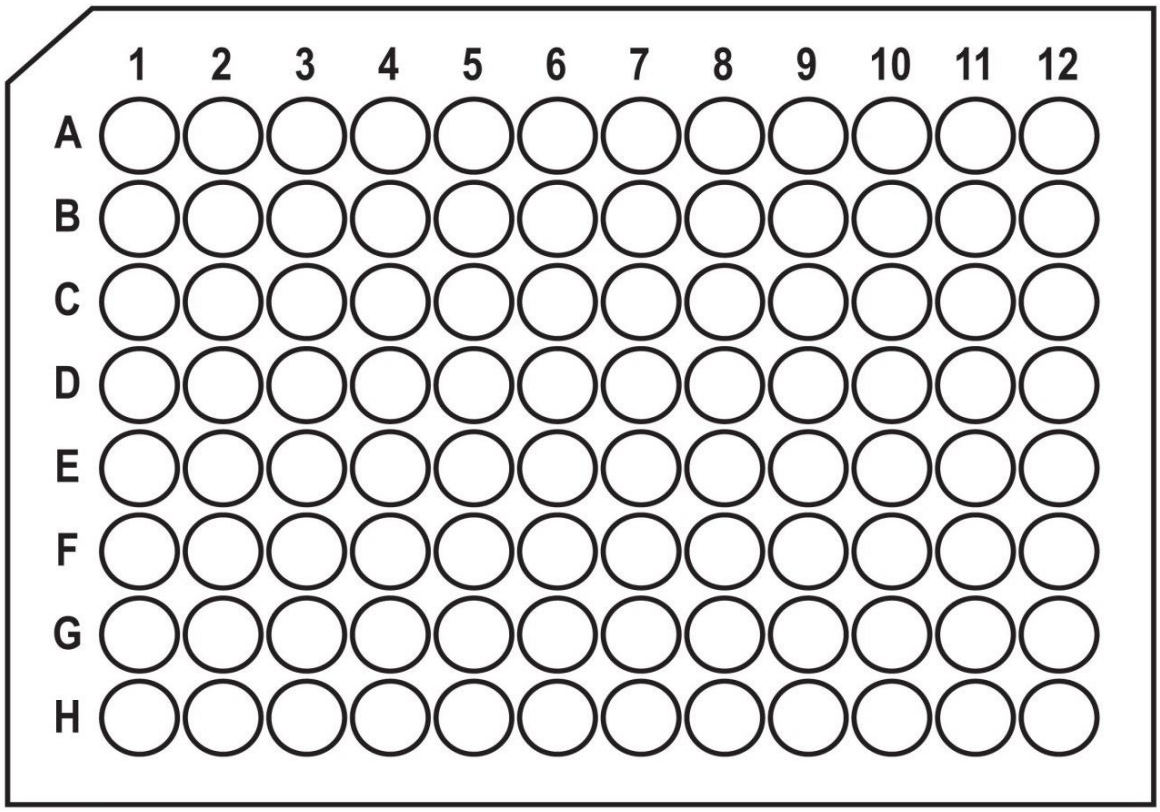

Figure 5. *Plate Diagram.*