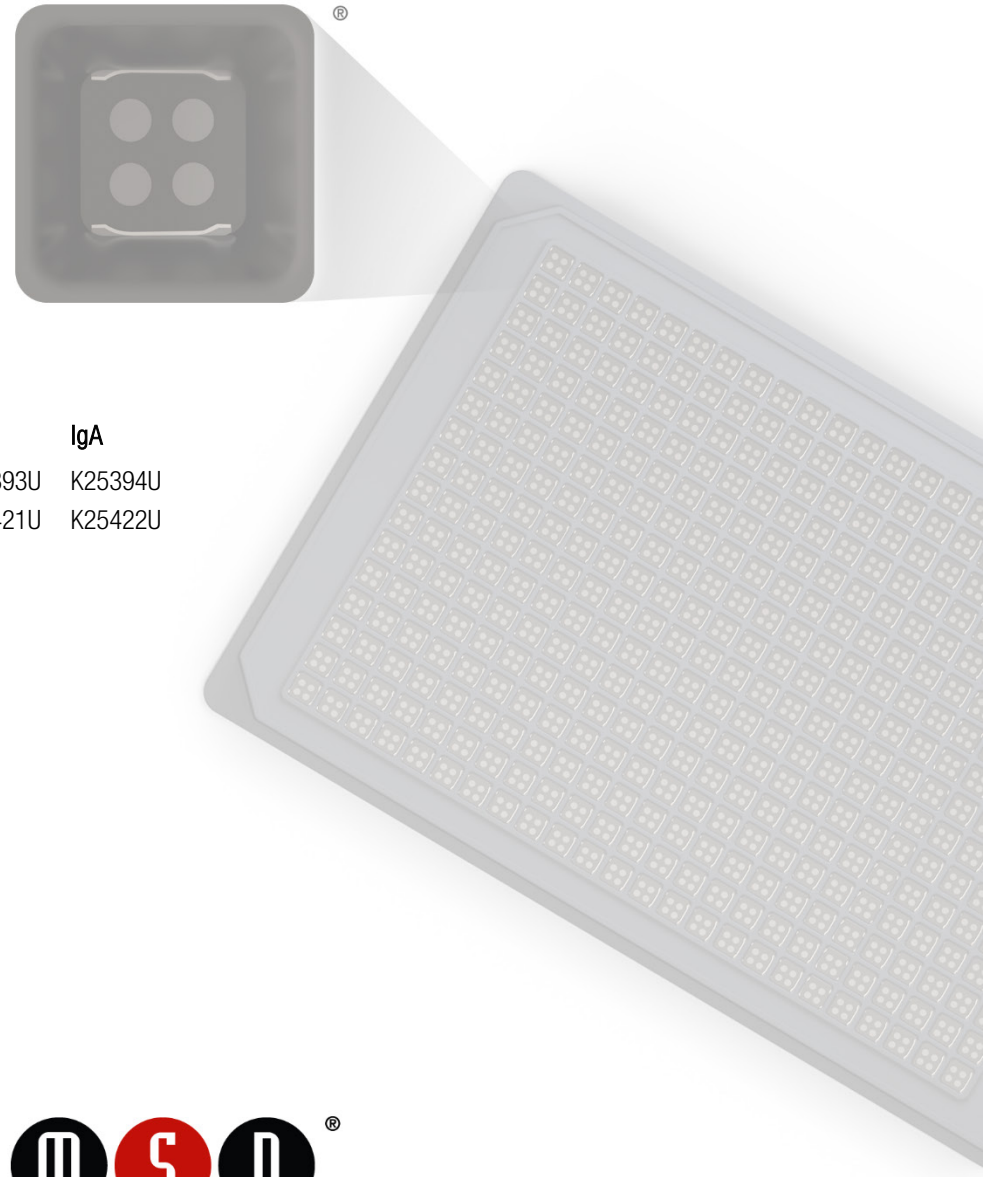
MSD® MULTI-SPOT Assay System

COVID-19 Serology Kits

V-PLEX®



V-PLEX® Serology Kits	IgG	IgM	IgA
SARS-CoV-2 384 Panel 1	K25392U	K25393U	K25394U
SARS-CoV-2 384 Panel 2	K25420U	K25421U	K25422U

V-PLEX COVID-19 Serology Kits

The V-PLEX COVID-19 Serology Kits for 384-well plate include multiple panels to detect antibodies to antigens from SARS CoV-2, including variants of the SARS-CoV-2 virus.

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

The V-PLEX COVID-19 Serology Kits for 384-well plate measure the presence and amount of antibodies to the SARS-CoV-2 virus. The kits are available as panels and detect isotypes (IgG, IgM, and IgA) of antigen-specific antibodies.

Principle of the Assay

The V-PLEX COVID-19 Serology Kits for 384-well plate quantitatively measure antibodies to antigens related to SARS-CoV-2, including variants of the SARS-CoV-2 virus. Plates are provided with antigens on spots in the well of a 384-well plate. Antibodies in the sample bind to the antigens on the spots, and anti-human antibodies (IgG, IgM, or IgA) conjugated with MSD SULFO-TAG™ are used for detection. The plate is read on an MSD® instrument, which measures the light emitted from the MSD SULFO-TAG.

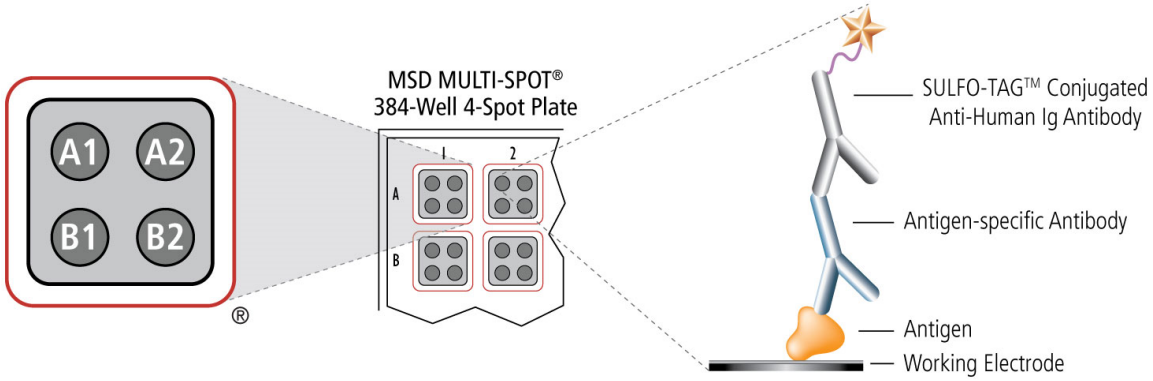

Figure 1. Schematic for V-PLEX COVID-19 Serology Kits for 384-well plate.

Kit Components

The V-PLEX COVID-19 Serology Kits for 384-well plate are available as panels defined by a set of antigens coated on a 4-spot MULTI-SPOT® 384-well plate. A kit includes a reference standard for quantitation, controls, plate(s), one of the available detection antibodies (anti-human IgG, IgM, or IgA), and all other reagents necessary to conduct the assay.

Table 1 describes the available plates and the location of antigens on each plate. Table 2 shows the relationship between the V-PLEX COVID-19 Serology Kits and the plates included in those kits. Together, Table 1 and Table 2 help users select the kits that contain their preferred antigen. Table 3 provides a list of components included in each kit.

Table 1. List of antigens and their spot assignments on the MULTI-SPOT 384-Well, 4-Spot plates

Plate Description	SARS-CoV-2 384 Plate 1	SARS-CoV-2 384 Plate 2
Spot A1	SARS-CoV-2 Spike	SARS-CoV-2 Spike
Spot A2	SARS-CoV-2 Nucleocapsid	SARS-CoV-2 Nucleocapsid
Spot B1	SARS-CoV-2 S1 RBD	SARS-CoV-2 S1 RBD (B.1.351)
Spot B2	*BSA*	SARS-CoV-2 Spike (B.1.351)

Table 2. Antigen plates included in V-PLEX COVID-19 Serology Kits for 384-well plate

Kit	Plate(s) Included
V-PLEX SARS-CoV-2 384 Panel 1 Kit	SARS-CoV-2 384 Plate 1
V-PLEX SARS-CoV-2 384 Panel 2 Kit	SARS-CoV-2 384 Plate 2

Table 3. Reagents and Components

Reagent	Storage	Catalog Number	Size	Quantity Supplied	
				5-Plate Kit	25-Plate Kit
MULTI-SPOT 384-Well, 4-Spot plate	2–8 °C	—	4-Spot	5 plates	25 plates
SULFO-TAG Anti-human IgG, IgM, or IgA Antibody (200X)	2–8 °C		200 µL		
SULFO-TAG Anti-Human IgG Antibody		D21ADF-3		2 vials	10 vials
SULFO-TAG Anti-Human IgM Antibody		D21ADD-3		2 vials	10 vials
SULFO-TAG Anti-Human IgA Antibody		D21ADE-3		2 vials	10 vials
Diluent 100	2–8 °C	R50AA-3	1000 mL	2 bottles	10 bottles
MSD Wash Buffer (20X)	RT	R61AA-1	100 mL	2 bottles	10 bottles
Blocker A	RT	R93BA-2	250 mL	1 bottle	5 bottles
MSD Phosphate Buffer (5X)	RT	R93SA-2	50 mL	1 bottle	5 bottles
MSD GOLD™ Read Buffer B	RT	R60AM-2	90 mL	1 bottle	5 bottles
Microplate Adhesive Film	RT	—	—	15 sheets	75 sheets
Reference Standard 1	≤-70 °C	C00ADK-2	1 mL	1 vial	5 vials
Serology Control Pack 1	≤-70 °C	C4381-1			
Serology Control 1.1			1 mL	1 vial	5 vials
Serology Control 1.2			1 mL	1 vial	5 vials
Serology Control 1.3			1 mL	1 vial	5 vials

RT = room temperature

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Deionized water
- ❑ 0.2 µM filter needed for Blocker A preparation
- ❑ Plate shaker capable of shaking at ~1500 rpm
- ❑ Microcentrifuge tubes for making serial dilutions
- ❑ Automated plate washer or other efficient multi-channel pipetting equipment for washing 384-well plates
- ❑ Appropriate liquid handling equipment for desired throughput capable of accurately dispensing 10 µL and 40 µL into a 384-well microplate
- ❑ Vortex mixer

Safety

Reference Standard 1 and Serology Control Pack 1 contain human serum and are biosafety level 2 (BSL-2) products. Donors have been tested and found negative for HBsAg, HIV-1 and HIV-2 antibodies, and HCV. Use safe laboratory practices and wear gloves, safety glasses, and laboratory coats when handling kit 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®.

Best Practices

- Mixing or substituting reagents from different sources or different kit lots is not recommended. A list of components and their lot numbers is included in the certificate of analysis (COA) of the kit.
- Assay incubation steps should be performed at 20–26 ºC to maximize consistency in signals between runs.
- Avoid prolonged exposure of the detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light except for direct sunlight.
- Avoid bubbles in wells at all pipetting steps. Bubbles may lead to variable results. Bubbles introduced when adding read buffer may interfere with signal detection.
- Do not touch the pipette tip on the bottom of the wells when pipetting into the MSD plate.
- Use reverse pipetting when necessary to avoid the introduction of bubbles. For empty wells, pipette to the bottom corner.
- Plate shaking should be vigorous, with a rotary motion around 1500 rpm.
- Gently tap the plate on a paper towel to remove residual fluid after washing.
- Avoid excessive drying of the plate during washing step. Add solutions to the plate immediately after washing.
- Read buffer should be at room temperature (20–26 ºC) when added to the plate.
- Keep time intervals consistent between adding read buffer and reading the plate to improve inter-plate precision. Unless otherwise directed, read plate as soon as possible after adding read buffer.
- Do not shake the plate after adding read buffer.
- Ensure that the reagents for the next step are prepared before washing the plates in order to prevent the plates from drying out.
- Remove the plate seals before reading the plate.
- If assay results are above the top of the calibration curve, dilute the samples and repeat the assay.
- We do not recommend attempting to use a partial plate when running this panel.

Recommended Protocol

Bring all plates and diluents to room temperature. Thaw samples, reference standard, and controls on ice. Thawed reference standard and controls should be equilibrated to room temperature before loading into the plates.

A sample plate layout is shown in Figure 4 (below).

Prepare Blocker A Solution

Follow the preparation procedure in the product insert provided with the Blocker A Kit to prepare the Blocker A solution. You may store unused Blocker A solution according to the instructions in the Blocker A product insert available at www.mesoscale.com.

Prepare Wash Buffer

MSD provides 100 mL of Wash Buffer as a 20X stock solution. Dilute the stock solution before use. PBS + 0.05% Tween-20 can be used as an alternative to MSD Wash Buffer.

For one plate, combine:

- ❑ 20 mL of MSD Wash Buffer (20X)
- ❑ 380 mL of deionized water

Assay Diluent

Use Diluent 100 as assay and antibody diluent.

STEP 1: Prepare Plate

- ❑ Remove the plate from its packaging.
- ❑ Add 50 µL/well of Blocker A solution to the plate.
- ❑ Seal the plate with an adhesive plate seal and incubate at room temperature without shaking for 1 hour.

During this time, prepare calibrators, controls, and samples.

Calibrator Preparation

The kits include a serum-based reference standard, Reference Standard 1, which is used to establish a calibration curve in the assay. The calibration curve is used for calculating the concentration of human IgG, IgM, and IgA against multiple antigens in the V-PLEX COVID-19 Serology Kits.

We recommend a 7-point calibration curve with 4-fold serial dilution steps and a zero calibrator blank. Thaw Reference Standard 1 on ice, equilibrate to room temperature, and then add to Diluent 100 to make the calibrator curve solutions.

Appendices A and B provide assigned values for calibrators in MSD arbitrary units (AU/mL) and WHO international units (BAU/mL).

Note: For the IgG assays, Reference Standard 1 requires a 10-fold dilution to create the highest calibrator point (CAL-01). Whereas, for the IgM and IgA assays, neat (undiluted) Reference Standard 1 stock solution should be used as the highest calibrator point (CAL-01).

CAL-01 Preparation: Reference Standard 1, 10-fold dilution:

Prepare the highest calibrator solution (CAL-01) for the **IgG assays** by diluting Reference Standard 1 **10-fold**, as shown below (Figure 2):

- ❑ Add 15 µL of the Reference Standard 1 to 135 µL of Diluent 100. Vortex briefly to mix. Label the vial as CAL-01.

CAL-01 Preparation: Reference Standard 1, Neat:

Use Reference Standard 1 without dilution (neat) as the highest calibrator solution (CAL-01) for the **IgM and IgA assays** (Figure 3).

- ❑ Vortex Reference Standard 1 briefly and spin down before opening the tube. Pipette 150 µL of the Reference Standard 1 into a tube and label as CAL-01.

CAL-02 to CAL-08 Preparation:

To prepare 7 additional calibrator solutions plus a zero calibrator for up to 2 replicates, perform the following:

- ❑ Prepare the next calibrator (CAL-02) by adding 40 µL of CAL-01 (IgG, IgM, or IgA) to 120 µL of Diluent 100. Vortex briefly to mix.
- ❑ Repeat 4-fold serial dilutions (40 µL previous calibrator into 120 µL Diluent 100) to generate CAL-03 through CAL-07.
- ❑ Use Diluent 100 as the blank (CAL-08).

Note: Stock calibrator is stable for 5 years from the date of manufacture when stored at ≤-70 °C. The thawed calibrator is stable through five freeze-thaw cycles. Excess diluted calibrator should be discarded after use.

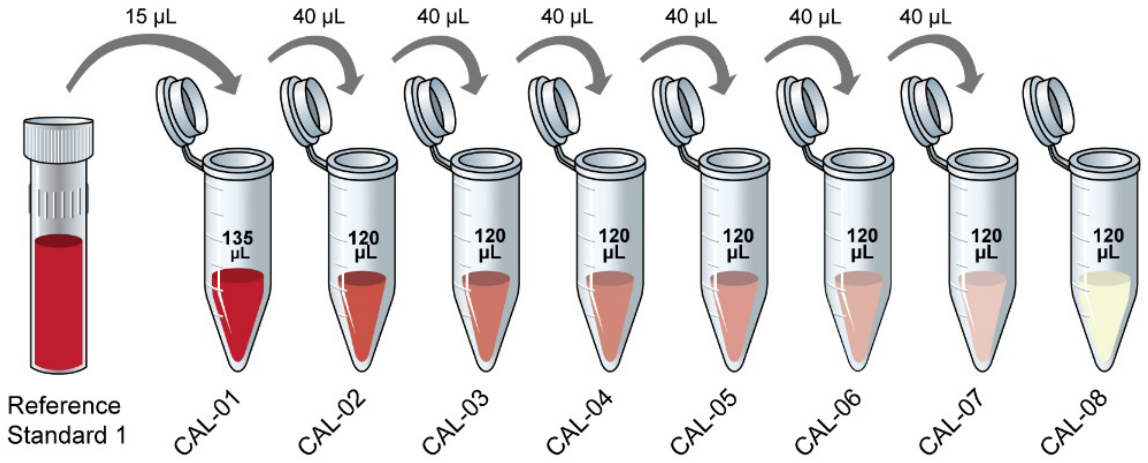

Figure 2. Dilution schema for preparation of calibrator solutions using a 10-fold dilution of the Reference Standard 1 to generate CAL-01.

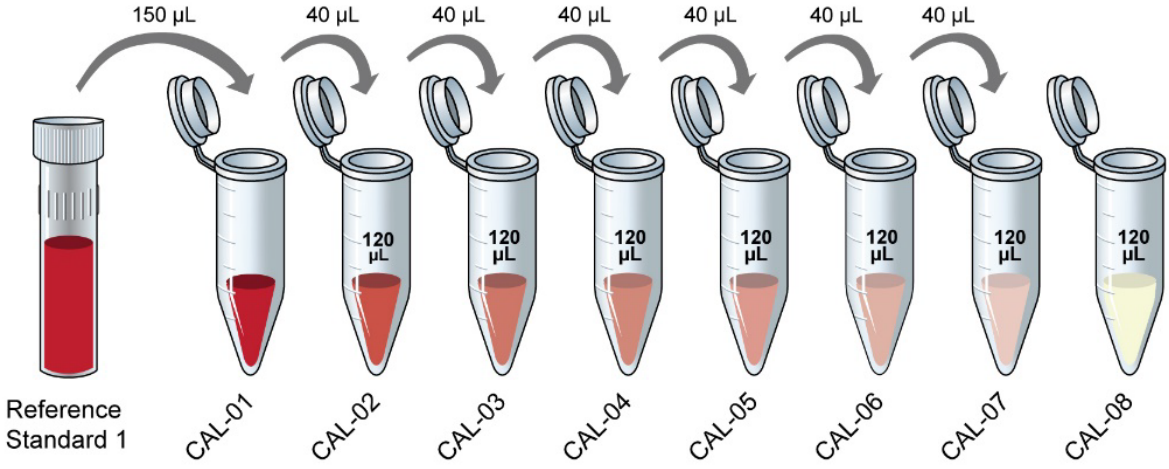

Figure 3. Dilution schema for preparation of calibrator solutions by using the Reference Standard 1 as neat (undiluted) to generate CAL-01.

Control Preparation

The Serology Control Pack consists of three levels of controls, each containing known concentrations of human IgG, IgM, and IgA in arbitrary units (AU/mL) against multiple antigens in the V-PLEX COVID-19 Serology Kits. Refer to **Appendix C** for the assigned values in MSD arbitrary units (AU/mL) and **Appendix D** for the assigned values in the WHO international units (BAU/mL).

Each control is supplied at the working concentration. Do not dilute prior to use.

Thaw the control on ice and equilibrate to room temperature. Vortex briefly and spin down before loading controls into the plate.

Note: Stock control is stable for 5 years from the date of manufacture when stored at ≤-70 °C. Thawed control is stable through five freeze-thaw cycles.

Sample Preparation

Prepare the samples by diluting with Diluent 100. The optimal dilution for serum and plasma samples should be determined empirically by the user. Typically, samples are measured at a dilution between 100-fold and 10,000-fold. The lower dilution keeps negative or low samples in the measurable range; higher dilutions prevent saturation of signal with strongly positive samples. This protocol provides guidance for preparing 100-fold and 5,000-fold dilutions, a common choice in vaccine and epidemiological studies.

Note: Saliva and upper respiratory samples are more variable in their composition than serum and plasma samples. Users should run a pilot dilution series to determine the optimal dilution for their saliva and upper respiratory samples.

This protocol provides guidance for preparing both a 100-fold and 5000-fold diluted sample.

1. To make an intermediate 1:10 dilution in a 2 mL tube, combine:
    - 10 µL of sample
    - 90 µL of Diluent 100
2. To make a 1:100 dilution in a 2 mL tube, combine:
    - 10 µL of the 1:10 dilution from Step 1.
    - 90 µL of Diluent 100
3. To make a 1:5,000 dilution in a 2 mL tube, combine:
    - 10 µL of the 1:100 dilution from Step 2.
    - 490 µL of Diluent 100

STEP 2: Calibrators, Controls, and Sample Addition

After the Blocker A incubation step, wash the plate 3 times with at least 90 µL/well of 1X MSD Wash buffer.

- ❑ Add 25 µL/well of diluted samples, calibrators, and controls to the plate.
- ❑ Seal the plate with an adhesive plate seal and incubate at room temperature with shaking (~1500 rpm) for 4 hours.

During this time, prepare detection antibody solution.

Detection Antibody Solution Preparation

Detection antibody is provided as a 200X stock solution. The working solution is 1X. You will need 12 mL per plate.

To prepare a 1X solution of detection antibody, combine:

- ❑ 11,940 µL of Diluent 100
- ❑ 60 µL of 200X SULFO-TAG anti-human Ig Antibody

STEP 3: Detection Antibody Addition

After the sample incubation step, wash the plate 3 times with at least 90 µL/well of 1X MSD Wash buffer.

- ❑ Add 25 µL/well of 1X detection antibody solution to the plate.
- ❑ Seal the plate with an adhesive plate seal and incubate at room temperature with shaking (~1500 rpm) for 1 hour.

STEP 4: Read Buffer Addition

After the detection antibody incubation step, wash the plate 3 times with at least 90 µL/well of 1X MSD Wash buffer.

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

- ❑ Add 40 µL/well of the MSD GOLD Read Buffer B to the plate.
- ❑ Read the plate on the MSD instrument. No incubation in read buffer is required before reading the plate. Read plate immediately after adding read buffer. Do not shake the plate after adding read buffer.

STEP 5: Analysis of Results

Calibration curves used to calculate antibody concentrations are established by fitting the signals from the calibrators to a 4-parameter logistic (or sigmoidal dose-response) model with a $1/Y^2$ weighting. Best quantification of unknown samples is achieved by generating a calibration curve for each plate using a minimum of two replicates at each calibrator level.

Antibody unit concentrations in controls and diluted samples are determined from their ECL signals by backfitting to the calibration curve.

For samples, correcting for dilution provides the final antibody concentrations in undiluted samples (in AU/ml). For example, if 1,000-fold diluted samples are tested, multiply the backfitted concentrations by 1,000.

Controls 1.1, 1.2, and 1.3 are provided pre-diluted for ease of use. Their assigned concentrations reflect the antibody concentrations in the as-provided material. Multiplying the backfitted concentrations of the controls by 5,000 will provide dilution-adjusted concentrations (in AU/mL) that are comparable to concentrations of antibodies in undiluted serum and plasma samples.

Calibration

Reference Standard 1 is calibrated against the WHO International Standard (NIBSC code: 20/136). To convert MSD assigned concentration units (AU/mL) to WHO/NIBSC units in binding antibody units (BAU/mL), use the equation below:

WHO/NIBSC International Standard Units (BAU/mL) = MSD Units (AU/mL) x conversion factor

Table 3. Calibration of MSD Reference Standard 1 (lot numbers A00V0004 and A0080270) against the WHO International Standard (NIBSC code: 20/136)

	IgG			IgM			IgA		
Antigens	MSD Units AU/mL	WHO Units BAU/mL	Conversion factor*	MSD Units AU/mL	WHO Units BAU/mL	Conversion factor*	MSD Units AU/mL	WHO Units BAU/mL	Conversion factor*
SARS-CoV-2 Nucleocapsid	800	1.89	0.00236	20	2.64	0.132	50	5.57	0.111
SARS-CoV-2 S1 RBD	300	8.16	0.0272	20	0.466	0.0233	20	1.56	0.0782
SARS-CoV-2 Spike	700	6.31	0.00901	40	0.867	0.0217	50	3.09	0.0619

* Conversion factor = WHO/NIBSC Units: MSD Units

Protocol at a Glance

Note: Bring all plates and diluents to room temperature. Thaw samples, reference standard, and controls on ice. Thawed reference standard and controls should be equilibrated to room temperature before loading into the plates.

- ❑ Add Blocker A solution; incubate without shaking for 1 hour, wash.
- ❑ Add samples, calibrators, and controls. Incubate for 4 hours and wash.
- ❑ Add Detection Antibody solution. Incubate for 1 hour and wash.
- ❑ Add Read Buffer and analyze plate.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	CAL-01		Sample-06		Sample-22		Sample-38		Sample-54		Sample-70		Sample-86		Sample-102		Sample-118		Sample-134		Sample-150		Sample-166	
B	CAL-02		Sample-07		Sample-23		Sample-39		Sample-55		Sample-71		Sample-87		Sample-103		Sample-119		Sample-135		Sample-151		Sample-167	
C	CAL-03		Sample-08		Sample-24		Sample-40		Sample-56		Sample-72		Sample-88		Sample-104		Sample-120		Sample-136		Sample-152		Sample-168	
D	CAL-04		Sample-09		Sample-25		Sample-41		Sample-57		Sample-73		Sample-89		Sample-105		Sample-121		Sample-137		Sample-153		Sample-169	
E	CAL-05		Sample-10		Sample-26		Sample-42		Sample-58		Sample-74		Sample-90		Sample-106		Sample-122		Sample-138		Sample-154		Sample-170	
F	CAL-06		Sample-11		Sample-27		Sample-43		Sample-59		Sample-75		Sample-91		Sample-107		Sample-123		Sample-139		Sample-155		Sample-171	
G	CAL-07		Sample-12		Sample-28		Sample-44		Sample-60		Sample-76		Sample-92		Sample-108		Sample-124		Sample-140		Sample-156		Sample-172	
H	CAL-08		Sample-13		Sample-29		Sample-45		Sample-61		Sample-77		Sample-93		Sample-109		Sample-125		Sample-141		Sample-157		Sample-173	
I	Control 1.1		Sample-14		Sample-30		Sample-46		Sample-62		Sample-78		Sample-94		Sample-110		Sample-126		Sample-142		Sample-158		Sample-174	
J	Control 1.2		Sample-15		Sample-31		Sample-47		Sample-63		Sample-79		Sample-95		Sample-111		Sample-127		Sample-143		Sample-159		Sample-175	
K	Control 1.3		Sample-16		Sample-32		Sample-48		Sample-64		Sample-80		Sample-96		Sample-112		Sample-128		Sample-144		Sample-160		Sample-176	
L	Sample-01		Sample-17		Sample-33		Sample-49		Sample-65		Sample-81		Sample-97		Sample-113		Sample-129		Sample-145		Sample-161		Sample-177	
M	Sample-02		Sample-18		Sample-34		Sample-50		Sample-66		Sample-82		Sample-98		Sample-114		Sample-130		Sample-146		Sample-162		Sample-178	
N	Sample-03		Sample-19		Sample-35		Sample-51		Sample-67		Sample-83		Sample-99		Sample-115		Sample-131		Sample-147		Sample-163		Sample-179	
O	Sample-04		Sample-20		Sample-36		Sample-52		Sample-68		Sample-84		Sample-100		Sample-116		Sample-132		Sample-148		Sample-164		Sample-180	
P	Sample-05		Sample-21		Sample-37		Sample-53		Sample-69		Sample-85		Sample-101		Sample-117		Sample-133		Sample-149		Sample-165		Sample-181	

Figure 4. Sample plate layout that can be used for the assay. Each sample, control, and calibrator is measured in duplicate in side-by-side wells.

Appendix A: Values for Reference Standard 1 in MSD Arbitrary units (AU/mL)

Assigned values for the concentration of IgG, IgM, and IgA antibodies in Reference Standard 1 (lot number A00V0004 and A0080270).

	IgG Concentration (AU/mL)							
Antigens	**CAL 01**	**CAL 02**	**CAL 03**	**CAL 04**	**CAL 05**	**CAL 06**	**CAL 07**	**CAL08**
SARS-CoV-2 Nucleocapsid	80	20	5.0	1.25	0.313	0.0781	0.0195	0
SARS-CoV-2 S1 RBD	30	7.5	1.88	0.469	0.117	0.0293	0.00732	0
SARS-CoV-2 Spike	70	17.5	4.38	1.09	0.273	0.0684	0.0171	0
SARS-CoV-2 S1 RBD (B.1.351)	6.3	1.58	0.394	0.0984	0.0246	0.00615	0.00154	0
SARS-CoV-2 Spike (B.1.351)	21	5.25	1.31	0.328	0.082	0.0205	0.00513	0

	IgM Concentration (AU/mL)							
Antigens	**CAL 01**	**CAL 02**	**CAL 03**	**CAL 04**	**CAL 05**	**CAL 06**	**CAL 07**	**CAL08**
SARS-CoV-2 Nucleocapsid	20	5.0	1.25	0.313	0.0781	0.0195	0.00488	0
SARS-CoV-2 S1 RBD	20	5.0	1.25	0.313	0.0781	0.0195	0.00488	0
SARS-CoV-2 Spike	40	10	2.5	0.625	0.156	0.0391	0.00977	0
SARS-CoV-2 S1 RBD (B.1.351)	1.7	0.425	0.106	0.0266	0.00664	0.00166	0.000415	0
SARS-CoV-2 Spike (B.1.351)	7.4	1.85	0.463	0.116	0.0289	0.00723	0.00181	0

	IgA Concentration (AU/mL)							
Antigens	**CAL 01**	**CAL 02**	**CAL 03**	**CAL 04**	**CAL 05**	**CAL 06**	**CAL 07**	**CAL08**
SARS-CoV-2 Nucleocapsid	50	12.5	3.13	0.781	0.195	0.0488	0.0122	0
SARS-CoV-2 S1 RBD	20	5.0	1.25	0.313	0.0781	0.0195	0.00488	0
SARS-CoV-2 Spike	50	12.5	3.13	0.781	0.195	0.0488	0.0122	0
SARS-CoV-2 S1 RBD (B.1.351)	3.4	0.85	0.213	0.0531	0.0133	0.00332	0.00083	0
SARS-CoV-2 Spike (B.1.351)	23	5.75	1.44	0.359	0.0898	0.0225	0.00562	0

Assigned values for the concentration of IgG, IgM, and IgA antibodies in Reference Standard 1 (lot number A0080286).

	IgG Concentration (AU/mL)							
Antigens	CAL 01	CAL 02	CAL 03	CAL 04	CAL 05	CAL 06	CAL 07	CAL08
SARS-CoV-2 Nucleocapsid	70	17.5	4.38	1.09	0.273	0.0684	0.0171	0
SARS-CoV-2 S1 RBD	29	7.25	1.81	0.453	0.113	0.0283	0.00708	0
SARS-CoV-2 Spike	72	18	4.5	1.13	0.281	0.0703	0.0176	0
SARS-CoV-2 S1 RBD (B.1.351)	4.4	1.1	0.275	0.0688	0.0172	0.0043	0.00107	0
SARS-CoV-2 Spike (B.1.351)	25	6.25	1.56	0.391	0.0977	0.0244	0.0061	0

	IgM Concentration (AU/mL)							
Antigens	CAL 01	CAL 02	CAL 03	CAL 04	CAL 05	CAL 06	CAL 07	CAL08
SARS-CoV-2 Nucleocapsid	49	12.3	3.06	0.766	0.191	0.0479	0.012	0
SARS-CoV-2 S1 RBD	98	24.5	6.13	1.53	0.383	0.0957	0.0239	0
SARS-CoV-2 Spike	130	32.5	8.13	2.03	0.508	0.127	0.0317	0
SARS-CoV-2 S1 RBD (B.1.351)	18	4.5	1.13	0.281	0.0703	0.0176	0.00439	0
SARS-CoV-2 Spike (B.1.351)	29	7.25	1.81	0.453	0.113	0.0283	0.00708	0

	IgA Concentration (AU/mL)							
Antigens	CAL 01	CAL 02	CAL 03	CAL 04	CAL 05	CAL 06	CAL 07	CAL08
SARS-CoV-2 Nucleocapsid	110	27.5	6.88	1.72	0.43	0.107	0.0269	0
SARS-CoV-2 S1 RBD	60	15	3.75	0.938	0.234	0.0586	0.0146	0
SARS-CoV-2 Spike	87	21.8	5.44	1.36	0.34	0.085	0.0212	0
SARS-CoV-2 S1 RBD (B.1.351)	6.2	1.55	0.388	0.0969	0.0242	0.00605	0.00151	0
SARS-CoV-2 Spike (B.1.351)	36	9.0	2.25	0.563	0.141	0.0352	0.00879	0

Appendix B: Values for Reference Standard 1 in WHO/NIBSC units (BAU/mL)

The WHO/NIBSC units (BAU/mL) for IgG, IgM, and IgA antibodies in Reference Standard 1 (lot number A00V0004 and A0080270).

	IgG Concentration (BAU/mL)							
Antigens	**CAL 01**	**CAL 02**	**CAL 03**	**CAL 04**	**CAL 05**	**CAL 06**	**CAL 07**	**CAL 08**
SARS-CoV-2 Nucleocapsid	0.189	0.0473	0.0118	0.00295	0.000738	0.000185	0.0000461	0
SARS-CoV-2 S1 RBD	0.816	0.204	0.051	0.0128	0.00319	0.000797	0.000199	0
SARS-CoV-2 Spike	0.631	0.158	0.039	0.00986	0.00246	0.000616	0.000154	0

	IgM Concentration (BAU/mL)							
Antigens	**CAL 01**	**CAL 02**	**CAL 03**	**CAL 04**	**CAL 05**	**CAL 06**	**CAL 07**	**CAL 08**
SARS-CoV-2 Nucleocapsid	2.64	0.66	0.165	0.0413	0.0103	0.00258	0.000645	0
SARS-CoV-2 S1 RBD	0.466	0.117	0.0291	0.00728	0.001820	0.000455	0.000114	0
SARS-CoV-2 Spike	0.868	0.217	0.0543	0.0136	0.00339	0.000848	0.000212	0

	IgA Concentration (BAU/mL)							
Antigens	**CAL 01**	**CAL 02**	**CAL 03**	**CAL 04**	**CAL 05**	**CAL 06**	**CAL 07**	**CAL 08**
SARS-CoV-2 Nucleocapsid	5.55	1.39	0.347	0.0867	0.0217	0.00542	0.00135	0
SARS-CoV-2 S1 RBD	1.56	0.39	0.0975	0.0244	0.00609	0.00152	0.000381	0
SARS-CoV-2 Spike	3.10	0.775	0.194	0.0484	0.0121	0.00303	0.000757	0

Appendix C: Values for Serology Controls in MSD Arbitrary units (AU/mL)

Assigned values for the concentration of IgG, IgM, and IgA antibodies in Serology Control 1.1 (lot number A00C0731), Serology Control 1.2 (lot number A00C0732), and Serology Control 1.3 (lot number A00C0733).

Antigen	Concentration of IgG (vialed)			Unit of Measure
	Serology Control 1.1	Serology Control 1.2	Serology Control 1.3	
SARS-CoV-2 Nucleocapsid	19.4	3.31	1.05	AU/mL
SARS-CoV-2 S1 RBD	7.18	2.12	0.545	AU/mL
SARS-CoV-2 Spike	16.7	4.13	1.48	AU/mL
SARS-CoV-2 S1 RBD (B.1.351)	1.41	0.49	0.174	AU/mL
SARS-CoV-2 Spike (B.1.351)	4.81	1.36	0.931	AU/mL

Antigen	Concentration of IgM (vialed)			Unit of Measure
	Serology Control 1.1	Serology Control 1.2	Serology Control 1.3	
SARS-CoV-2 Nucleocapsid	0.462	0.227	0.216	AU/mL
SARS-CoV-2 S1 RBD	0.474	0.20	0.292	AU/mL
SARS-CoV-2 Spike	0.863	0.25	0.356	AU/mL
SARS-CoV-2 S1 RBD (B.1.351)	0.0494	0.0583	0.0797	AU/mL
SARS-CoV-2 Spike (B.1.351)	0.174	0.0548	0.118	AU/mL

Antigen	Concentration of IgA (vialed)			Unit of Measure
	Serology Control 1.1	Serology Control 1.2	Serology Control 1.3	
SARS-CoV-2 Nucleocapsid	1.27	0.213	0.34	AU/mL
SARS-CoV-2 S1 RBD	0.504	0.167	0.109	AU/mL
SARS-CoV-2 Spike	1.24	0.371	0.166	AU/mL
SARS-CoV-2 S1 RBD (B.1.351)	0.0709	0.027	0.0144	AU/mL
SARS-CoV-2 Spike (B.1.351)	0.496	0.150	0.0614	AU/mL

Assigned values for the concentration of IgG, IgM, and IgA antibodies in Serology Control 1.1 (lot number A00C0771), Serology Control 1.2 (lot number A00C0772), and Serology Control 1.3 (lot number A00C0773).

Antigen	Concentration of IgG (vialed)			Unit of Measure
	Serology Control 1.1	Serology Control 1.2	Serology Control 1.3	
SARS-CoV-2 Nucleocapsid	22.9	7.59	4.54	AU/mL
SARS-CoV-2 S1 RBD	9.54	3.42	0.85	AU/mL
SARS-CoV-2 Spike	22.7	9.74	2.19	AU/mL
SARS-CoV-2 S1 RBD (B.1.351)	1.35	0.552	0.173	AU/mL
SARS-CoV-2 Spike (B.1.351)	7.12	3.31	0.798	AU/mL

Antigen	Concentration of IgM (vialed)			Unit of Measure
	Serology Control 1.1	Serology Control 1.2	Serology Control 1.3	
SARS-CoV-2 Nucleocapsid	1.34	0.322	0.472	AU/mL
SARS-CoV-2 S1 RBD	3.13	0.691	0.457	AU/mL
SARS-CoV-2 Spike	3.70	1.21	0.614	AU/mL
SARS-CoV-2 S1 RBD (B.1.351)	<LLOQ	<LLOQ	<LLOQ	AU/mL
SARS-CoV-2 Spike (B.1.351)	0.88	0.437	0.093	AU/mL

<LLOQ = below the lower limit of quantification (see appendix E)

Antigen	Concentration of IgA (vialed)			Unit of Measure
	Serology Control 1.1	Serology Control 1.2	Serology Control 1.3	
SARS-CoV-2 Nucleocapsid	3.44	0.185	0.875	AU/mL
SARS-CoV-2 S1 RBD	1.85	0.285	0.975	AU/mL
SARS-CoV-2 Spike	2.68	0.609	0.877	AU/mL
SARS-CoV-2 S1 RBD (B.1.351)	0.201	0.04	0.043	AU/mL
SARS-CoV-2 Spike (B.1.351)	1.12	0.391	0.161	AU/mL

Appendix D: Values for Serology Controls in WHO/NIBSC units (BAU/mL)

The WHO/NIBSC units (BAU/mL) for IgG, IgM, and IgA antibodies in Serology Control 1.1 (lot number A00C0731), Serology Control 1.2 (lot number A00C0732), and Serology Control 1.3 (lot number A00C0733).

Antigen	Concentration of IgG (vialed)			Unit of Measure
	Serology Control 1.1	Serology Control 1.2	Serology Control 1.3	
SARS-CoV-2 Nucleocapsid	0.0457	0.0078	0.0025	BAU/mL
SARS-CoV-2 S1 RBD	0.1952	0.0576	0.0148	BAU/mL
SARS-CoV-2 Spike	0.1504	0.0372	0.0133	BAU/mL

Antigen	Concentration of IgM (vialed)			Unit of Measure
	Serology Control 1.1	Serology Control 1.2	Serology Control 1.3	
SARS-CoV-2 Nucleocapsid	0.061	0.030	0.0285	BAU/mL
SARS-CoV-2 S1 RBD	0.011	0.0047	0.0068	BAU/mL
SARS-CoV-2 Spike	0.0187	0.0054	0.0077	BAU/mL

Antigen	Concentration of IgA (vialed)			Unit of Measure
	Serology Control 1.1	Serology Control 1.2	Serology Control 1.3	
SARS-CoV-2 Nucleocapsid	0.1414	0.0237	0.0379	BAU/mL
SARS-CoV-2 S1 RBD	0.0394	0.0131	0.0085	BAU/mL
SARS-CoV-2 Spike	0.0768	0.023	0.0103	BAU/mL

The WHO/NIBSC units (BAU/mL) for IgG, IgM, and IgA antibodies in Serology Control 1.1 (lot number A00C0771), Serology Control 1.2 (lot number A00C0772), and Serology Control 1.3 (lot number A00C0773).

Antigen	Concentration of IgG (vialed)			Unit of Measure
	Serology Control 1.1	Serology Control 1.2	Serology Control 1.3	
SARS-CoV-2 Nucleocapsid	0.054	0.0179	0.0107	BAU/mL
SARS-CoV-2 S1 RBD	0.2595	0.093	0.0231	BAU/mL
SARS-CoV-2 Spike	0.2045	0.0878	0.0197	BAU/mL

Antigen	Concentration of IgM (vialed)			Unit of Measure
	Serology Control 1.1	Serology Control 1.2	Serology Control 1.3	
SARS-CoV-2 Nucleocapsid	0.1769	0.0425	0.0623	BAU/mL
SARS-CoV-2 S1 RBD	0.0729	0.0161	0.0106	BAU/mL
SARS-CoV-2 Spike	0.0803	0.0263	0.0133	BAU/mL

Antigen	Concentration of IgA (vialed)			Unit of Measure
	Serology Control 1.1	Serology Control 1.2	Serology Control 1.3	
SARS-CoV-2 Nucleocapsid	0.3818	0.0205	0.0971	BAU/mL
SARS-CoV-2 S1 RBD	0.1447	0.0223	0.0762	BAU/mL
SARS-CoV-2 Spike	0.1659	0.0377	0.0543	BAU/mL

Appendix E: Sensitivity

Limits of quantification (LOQ) were first estimated based on the Reference Standard 1 performance over multiple runs. The LLOQ (lower limit of quantification) and ULOQ (upper limit of quantification) were determined by diluting the Reference Standard 1 in assay diluent to the target estimated LOQ levels and assessing the accuracy (70% to 130%) and precision (concentration CVs <20%) of the LOQ samples. The table below shows the in-well quantitative range for each assay. Multiplying the LLOQ and ULOQ values in the table by the sample dilution factor will provide dilution-adjusted limits of quantification.

	LLOQ and ULOQ concentration in MSD arbitrary units (AU/mL)					
	IgG		IgM		IgA	
Antigen	LLOQ	ULOQ	LLOQ	ULOQ	LLOQ	ULOQ
SARS-CoV-2 Nucleocapsid	0.046	80	0.083	5.0	0.060	50
SARS-CoV-2 S1 RBD	0.035	30	0.071	8.0	0.140	20
SARS-CoV-2 Spike	0.049	70	0.123	8.0	0.062	50
SARS-CoV-2 S1 RBD (B.1.351)	0.053	6.3	NA*	NA*	0.035	3.4
SARS-CoV-2 Spike (B.1.351)	0.072	21	0.054	7.4	0.057	23

NA*= not assigned

Appendix F: Clinical Sensitivity and Specificity

Clinical sensitivity, specificity, and cutoff values were established for three SARS-CoV-2 antigens using receiver operating characteristic curve (ROC) analysis. Commercially sourced serum samples from pre-2019 healthy adults (N=200) and PCR-confirmed COVID-19 patients (N=214) were tested. PCR-positive samples were grouped by time from diagnosis: 0 to 14 and 15+ days. The cutoff values shown in the table below were determined based on samples run at 5,000-fold dilution.

	IgG				
Antigen	Cutoff Value*	Units	Early Sensitivity (Day 0-14)‡	Late Sensitivity (Day 15+)‡	Specificity‡
SARS-CoV-2 Nucleocapsid	5,000	AU/mL	71.1% (54.1%–84.6%)	93.8% (89.1%–96.8%)	100.0% (98.2%–100%)
SARS-CoV-2 S1 RBD	538	AU/mL	71.1% (54.1%–84.6%)	98.3% (95.1%–99.6%)	98.5% (95.7%–99.7%)
SARS-CoV-2 Spike	1,960	AU/mL	84.2% (68.7%–94.0%)	98.3% (95.1%–99.6%)	99.5% (97.2%–100%)

*Dilution-adjusted sample concentration. Cutoff values provided for RUO purposes only

‡95% Confidence Interval shown in parenthesis

Appendix G

The tables below provide detailed information about the antigen coated on the plate and detection antibody source and clonality.

Coated Antigens

Antigens	Antigen Description*	Antigen Modifications
SARS-CoV-2 Nucleocapsid	Severe Acute Respiratory Syndrome Coronavirus 2 Nucleocapsid Protein	Full length Nucleocapsid; C-terminal His-Tag
SARS-CoV-2 S1 RBD	Severe Acute Respiratory Syndrome Coronavirus 2 Receptor Binding Domain of the S1 subunit	R319-F541 of the SARS-2 CoV Spike Sequence; C-terminal His-Tag
SARS-CoV-2 Spike	Severe Acute Respiratory Syndrome Coronavirus 2 Spike Protein	Soluble ectodomain with T4 trimerization domain; C-terminal Strep-Tag and His-Tag
SARS-CoV-2 S1 RBD (B.1.351)	Severe Acute Respiratory Syndrome Coronavirus 2 Receptor Binding Domain of the S1 subunit South Africa variant B.1.351 lineage	R319-F541 of the SARS-2 CoV Spike Sequence; C-terminal His-Tag; K417N, E484K, N501Y
SARS-CoV-2 Spike (B.1.351)	Severe Acute Respiratory Syndrome Coronavirus 2 Spike Protein South Africa variant B.1.351 lineage	Soluble ectodomain with T4 trimerization domain; C-terminal Strep-Tag and His-Tag; L18F, D80A, D215G, Δ242-244, R246I, K417N, E484K, N501Y, D614G, A701V

**EXPI293 cell line used as an expression system*

Antibodies

Antibody	Source Species
IgG	Mouse monoclonal
IgM	Mouse monoclonal
IgA	Mouse monoclonal

Catalog Numbers

Table 5. Catalog Number for V-PLEX COVID-19 Serology Kits for 384-well plate

Kit Name	IgG		IgM		IgA	
	5-Plate Kit	25-Plate Kit	5-Plate Kit	25-Plate Kit	5-Plate Kit	25-Plate Kit
Multiplex Kits on the MULTI-SPOT 384-Well, 4-Spot plate						
V-PLEX SARS-CoV-2 384 Panel 1 Serology Kit	K25392U-2	K25392U-4	K25393U-2	K25393U-4	K25394U-2	K25394U-4
V-PLEX SARS-CoV-2 384 Panel 2 Serology Kit	K25420U-2	K25420U-4	K25421U-2	K25421U-4	K25422U-2	K25422U-4

Plate Diagram

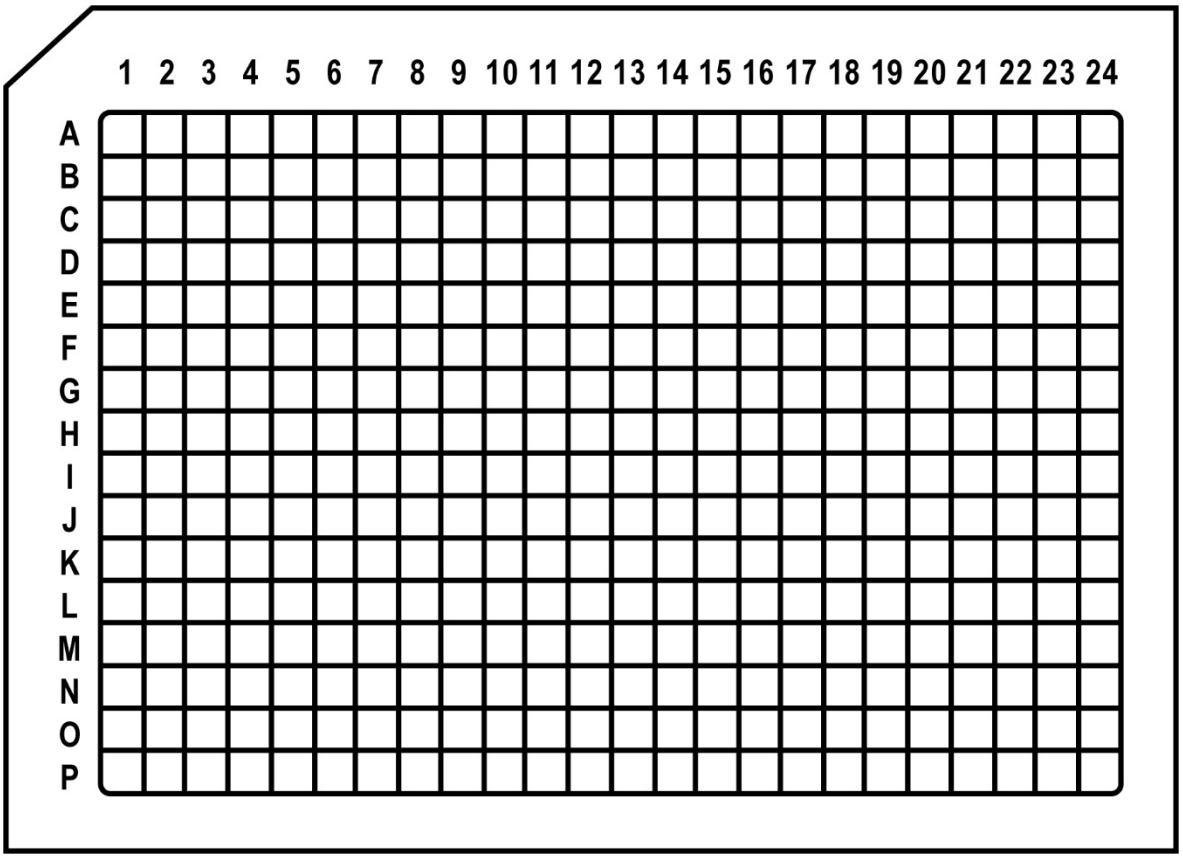

Figure 5. Plate diagram.