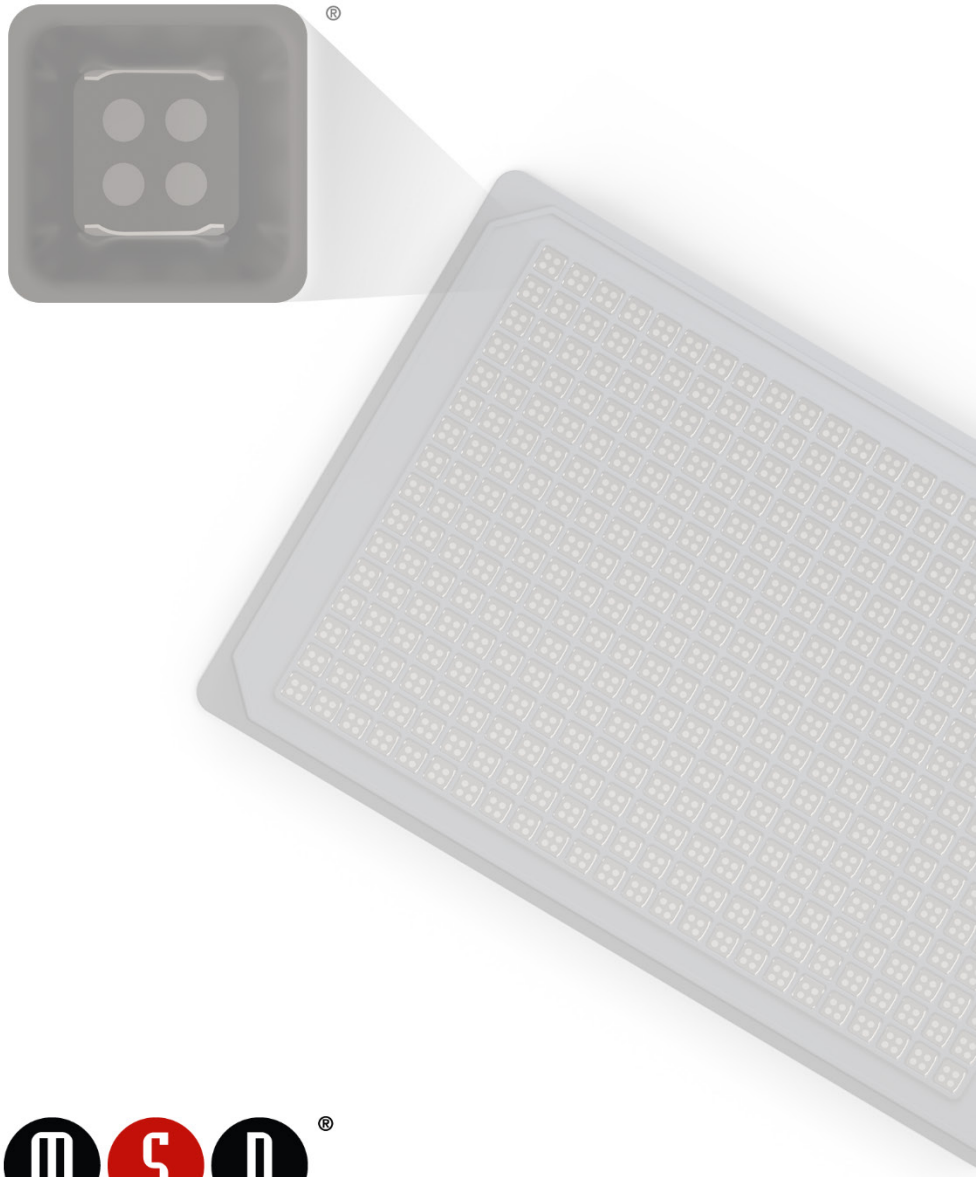
MSD® MULTI-SPOT Assay System

COVID-19 ACE2 Neutralization Kits

V-PLEX®



V-PLEX® Neutralization Kits	ACE2
SARS-CoV-2 384 Panel 1	K25395U
SARS-CoV-2 384 Panel 2	K25423U

V-PLEX COVID-19 ACE2 Neutralization Kits

The V-PLEX COVID-19 ACE2 Neutralization Kits for 384-well plate include multiple panels to measure antibodies that block the binding of angiotensin-converting enzyme 2 (ACE2) to the SARS-CoV-2 Spike and RBD antigens, 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 ACE2 Neutralization Kits for 384-well plate measure antibodies that block the binding of angiotensin-converting enzyme 2 (ACE2) to the SARS-CoV-2 Spike and RBD antigens, including variants of the SARS-CoV-2 virus. The assay serves as a high-throughput alternative to traditional neutralization assays.

Principle of the Assay

The V-PLEX COVID-19 ACE2 Neutralization Kits for 384-well plate quantitatively measure antibodies that block the binding of ACE2 to its cognate ligands (Table 1 and 2). Plates are provided with antigens on spots in the wells of a 384-well plate (Figure 1). Blocking antibodies in the sample bind to antigens on the spots, and human ACE2 protein conjugated with MSD SULFO-TAG™ is used for detection. The plate is read on an MSD® instrument, which measures the light emitted from the MSD SULFO-TAG.

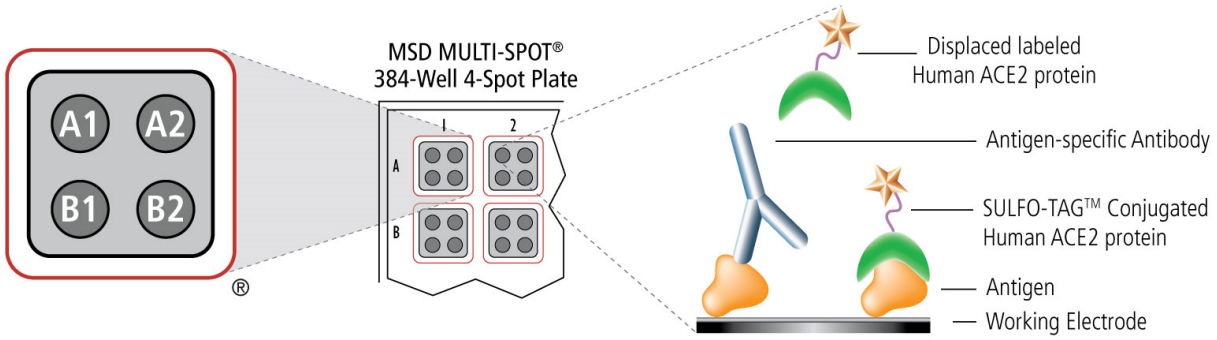

Figure 1. *Schematic for V-PLEX COVID-19 ACE2 Neutralization Kits for 384-well plate.*

Kit Components

The V-PLEX COVID-19 ACE2 Neutralization Kits for 384-well plate are available as panels defined by a set of viral antigens coated on a 4-spot MULTI-SPOT® 384-well plate. A kit includes a calibration reagent for quantification, human ACE2 protein as detection reagent, plate(s), 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 ACE2 Neutralization 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 ACE2 Neutralization 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 Human ACE2 Protein (200X)	2–8 °C*	D21ADG-3	100 µL	2 vials	10 vials
ACE2 Calibration Reagent (10X)	2–8 °C**	C01ADG-2	65 µL	1 vial	5 vials
Diluent 100	2–8 °C	R50AA-2	200 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 bottle
MSD Phosphate Buffer (5X)	RT	R93SA-2	50 mL	1 bottle	5 bottle
MSD GOLD™ Read Buffer B	RT	R60AM-2	90 mL	1 bottle	5 bottle
Microplate Adhesive Film	RT	—	—	15 sheets	75 sheets

RT = room temperature

*Store at 2-8°C with an expiration of 24 months from the date of manufacture

** Store at 2-8°C with an expiration of 42 months from the date of manufacture

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

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 ACE2 protein (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 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, calibration reagent, and diluents to room temperature. Thaw samples on ice.

A sample plate layout is shown in Figure 3 (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 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 samples and calibrators.

Sample Preparation:

Prepare the samples by diluting with Diluent 100. The optimal dilution for samples should be determined empirically by the user. Typically, serum and plasma samples are measured at a dilution between 10-fold and 100-fold to keep samples within the measurable range.

Note: For other sample types, users should run a pilot dilution series to determine the optimal dilution.

This protocol provides guidance for preparing 10-fold and 100-fold dilutions.

1. To make an intermediate 1:10 dilution in a 1.5 mL tube, combine:
    - ❑ 10 µL of sample
    - ❑ 90 µL of Diluent 100
2. To make a 1:100 dilution in a 1.5 mL tube, combine:
    - ❑ 10 µL of the 1:10 dilution from Step 1.
    - ❑ 90 µL of Diluent 100

Calibrator Preparation:

The kits include a Calibration Reagent, which is used to establish a calibration curve in the assay. Calibration Reagent for the competition assay (COVID-19 neutralizing antibody) is supplied at a 10-fold higher concentration than the recommended highest calibrator. We recommend a 7-point calibration curve with 4-fold serial dilution steps and a zero calibrator blank (Figure 2). Equilibrate the 10X calibrator stock to room temperature, and then add to Diluent 100 to make the calibration curve solution. Table 4 below shows the concentrations at each calibrator point.

Table 4. Calibrator fold-dilution

Standard	Dilution (-Fold)	Concentration (unit/mL)*
10X Calibrator Stock.	NA	200
CAL-01	10	20.0
CAL-02	4	5.00
CAL-03	4	1.25
CAL-04	4	0.312
CAL-05	4	0.0781
CAL-06	4	0.0195
CAL-07	4	0.00488
CAL-08	NA	0

NA = not applicable
* 1 unit/mL corresponds to neutralizing activity of 1 ug/mL monoclonal antibody to SARS CoV-2 Spike Protein

Volumes below are enough for up to 2 replicates.

1. To make the CAL-01 in a 2 mL tube, combine:
   - ❑ 10 µL of the 10X Calibrator Stock
   - ❑ 90 µL of Diluent 100
2. To prepare a 4-fold dilution for the next calibrator, CAL-02, in a 2 mL tube combine:
   - ❑ 25 µL of the diluted calibrator from Step 1
   - ❑ 75 µL of Diluent 100
3. Repeat 4-fold serial dilutions 5 additional times to generate 6 calibrators
4. The 8th standard CAL-08 is Diluent 100 (zero calibrator)

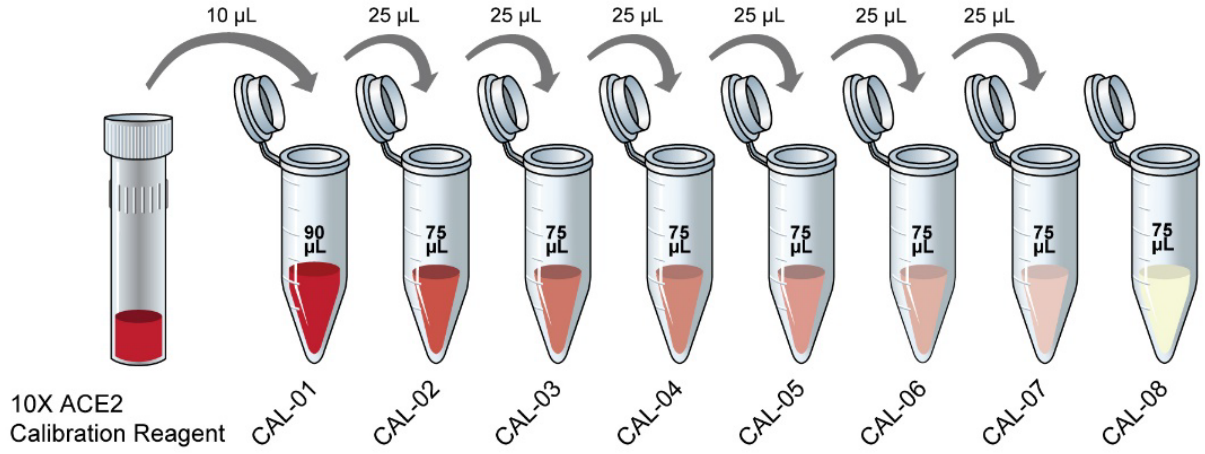

Figure 2. Dilution schema for preparation of calibrator solutions.

STEP 2: Sample and Calibrator Addition

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

- ❑ Add 15 µL/well of diluted samples and calibrator to the plate.
- ❑ Seal the plate with an adhesive plate seal and incubate at room temperature with shaking (~1500 rpm) for 4 hours.

Note: **Do not aspirate or wash the plate prior to addition of detection solution.**

During this time, prepare the ACE2 detection solution.

ACE2 Detection Solution Preparation:

SULFO-TAG Human ACE2 Protein is provided as a 200X stock solution. The working solution is 1X. You will need 7 mL per plate. To prepare a 1X solution of SULFO-TAG Human ACE2 Protein, combine:

- ❑ 6,965 µL of Diluent 100
- ❑ 35 µL of 200X SULFO-TAG Human ACE2 Protein

STEP 3: ACE2 Detection Addition

After the sample and calibrator incubation, **do not aspirate or wash the plate prior to addition of detection solution.**

- ❑ Add 15 µL/well of 1X SULFO-TAG Human ACE2 Protein detection 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 detect 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.
- ❑ 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

Results can be reported as percent inhibition, calculated using the equation below. Highly positive samples show high percent inhibition whereas negative or low samples show low percent inhibition.

$$\%\ Inhibition = 1 - \frac{Average\ Sample\ ECL\ Signal}{Average\ ECL\ signal\ of\ Calibrator\ 8\ (Diluent\ only)}\ x\ 100$$

Alternatively, the calibration curve can be used to calculate neutralizing antibody concentrations in samples, by backfitting the measured signals for samples to the calibration curve. Correcting for dilution provides the final neutralizing antibody concentrations in undiluted samples (in unit/ml). For example, if 100-fold diluted samples are tested, multiply the backfitted concentrations by 100.

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.

Note: To allow accurate and meaningful comparison between samples, compare results obtained using the same sample dilution.

Protocol at a Glance

Note: Bring all plates, calibration reagent, and diluents to room temperature. Thaw samples on ice.

- ❑ Add Blocker A solution; incubate without shaking for 1 hour, wash.
- ❑ Add samples and calibrators; incubate for 4 hours, do **not** wash.
- ❑ Add Detection ACE2 solution; incubate for 1 hour, 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-09		Sample-25		Sample-41		Sample-57		Sample-73		Sample-89		Sample-105		Sample-121		Sample-137		Sample-153		Sample-169	
B	CAL-02		Sample-10		Sample-26		Sample-42		Sample-58		Sample-74		Sample-90		Sample-106		Sample-122		Sample-138		Sample-154		Sample-170	
C	CAL-03		Sample-11		Sample-27		Sample-43		Sample-59		Sample-75		Sample-91		Sample-107		Sample-123		Sample-139		Sample-155		Sample-171	
D	CAL-04		Sample-12		Sample-28		Sample-44		Sample-60		Sample-76		Sample-92		Sample-108		Sample-124		Sample-140		Sample-156		Sample-172	
E	CAL-05		Sample-13		Sample-29		Sample-45		Sample-61		Sample-77		Sample-93		Sample-109		Sample-125		Sample-141		Sample-157		Sample-173	
F	CAL-06		Sample-14		Sample-30		Sample-46		Sample-62		Sample-78		Sample-94		Sample-110		Sample-126		Sample-142		Sample-158		Sample-174	
G	CAL-07		Sample-15		Sample-31		Sample-47		Sample-63		Sample-79		Sample-95		Sample-111		Sample-127		Sample-143		Sample-159		Sample-175	
H	CAL-08		Sample-16		Sample-32		Sample-48		Sample-64		Sample-80		Sample-96		Sample-112		Sample-128		Sample-144		Sample-160		Sample-176	
I	Sample-01		Sample-17		Sample-33		Sample-49		Sample-65		Sample-81		Sample-97		Sample-113		Sample-129		Sample-145		Sample-161		Sample-177	
J	Sample-02		Sample-18		Sample-34		Sample-50		Sample-66		Sample-82		Sample-98		Sample-114		Sample-130		Sample-146		Sample-162		Sample-178	
K	Sample-03		Sample-19		Sample-35		Sample-51		Sample-67		Sample-83		Sample-99		Sample-115		Sample-131		Sample-147		Sample-163		Sample-179	
L	Sample-04		Sample-20		Sample-36		Sample-52		Sample-68		Sample-84		Sample-100		Sample-116		Sample-132		Sample-148		Sample-164		Sample-180	
M	Sample-05		Sample-21		Sample-37		Sample-53		Sample-69		Sample-85		Sample-101		Sample-117		Sample-133		Sample-149		Sample-165		Sample-181	
N	Sample-06		Sample-22		Sample-38		Sample-54		Sample-70		Sample-86		Sample-102		Sample-118		Sample-134		Sample-150		Sample-166		Sample-182	
O	Sample-07		Sample-23		Sample-39		Sample-55		Sample-71		Sample-87		Sample-103		Sample-119		Sample-135		Sample-151		Sample-167		Sample-183	
P	Sample-08		Sample-24		Sample-40		Sample-56		Sample-72		Sample-88		Sample-104		Sample-120		Sample-136		Sample-152		Sample-168		Sample-184	

Figure 3. *Sample plate layout that can be used for the assay. Each sample and calibrator is measured in duplicate in side-by-side wells.*

Appendix A: Clinical Sensitivity and Specificity

Clinical sensitivity, specificity, and cutoff values were established for two 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 10-fold dilution.

	ACE2				
Antigen	Cutoff Value*	Units	Early Sensitivity (Day 0-14)‡	Late Sensitivity (Day 15+)‡	Specificity‡
SARS-CoV-2 S1 RBD	0.726	µg/mL	86.8% (71.9%–95.6%)	97.7% (94.3%–99.4%)	98.5% (95.7%–99.7%)
SARS-CoV-2 Spike	0.533	µg/mL	92.1% (78.6%–98.3%)	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 B

The table below provides 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 expression system*

Calibrator Reagent

SARS-CoV/SARS-CoV-2 Spike Monoclonal Antibody.

Detection Reagent

Recombinant Human Angiotensin-Converting Enzyme 2 (ACE-2).

Catalog Numbers

Table 5. *Catalog Number for V-PLEX COVID-19 ACE2 Neutralization Kits for 384-well plate*

Kit Name	ACE2	
	5-Plate Kit	25-Plate Kit
V-PLEX SARS-CoV-2 384 Panel 1 Kit	K25395U-2	K25395U-4
V-PLEX SARS-CoV-2 384 Panel 2 Kit	K25423U-2	K25423U-4

Plate Diagram

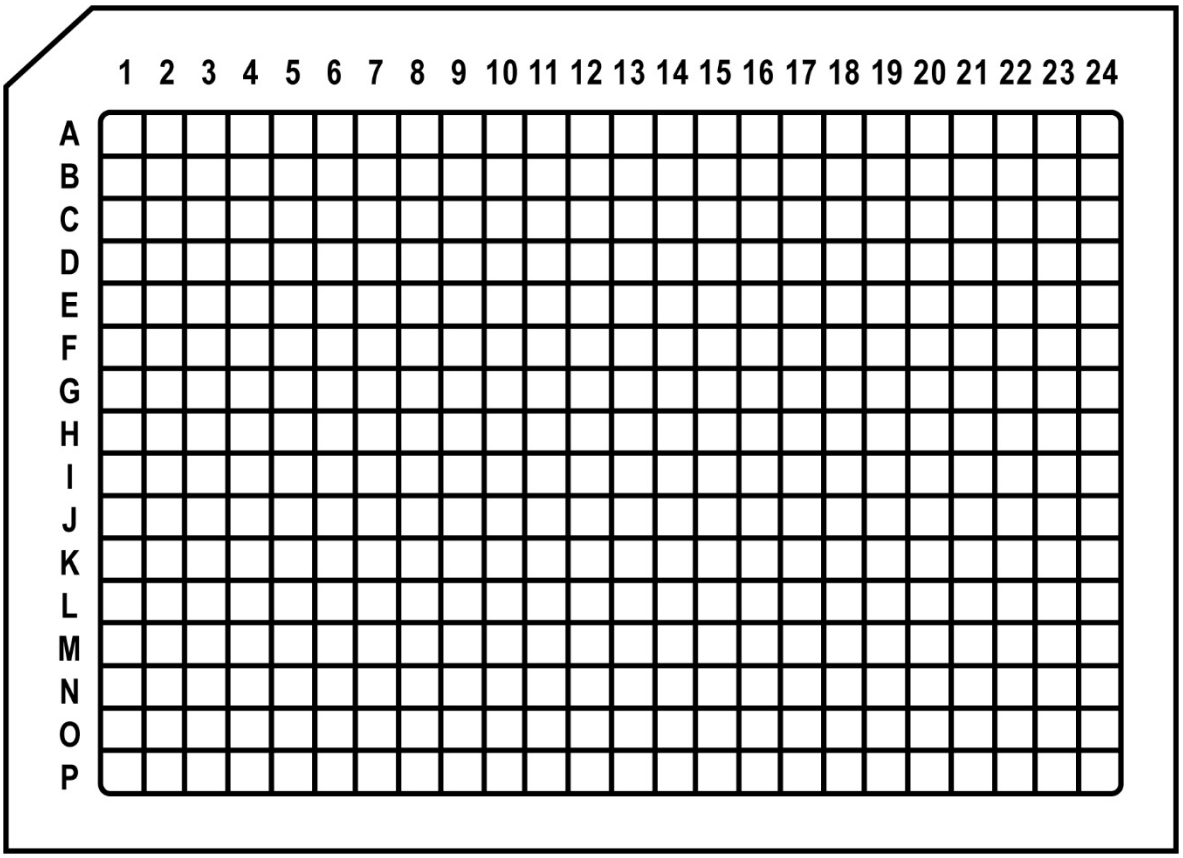

Figure 4. Plate diagram.