SARS-CoV-2 Variant Inhibitor Screening Kit

Catalog Number VANC00B

For evaluation of blocking antibodies in donor samples or inhibitors for screening of SARS-CoV-2 WT or variant Spike RBD protein association with ACE-2.

This kit may be used with either a single or any combination of the included WT and variant Spike RBD protein(s) depending on research needs.

This kit contains sufficient materials to run at least five 96 well plates, provided the following conditions are met:

- The reagents are prepared as described in this package insert.
- The assay is run as described in the Assay Procedure.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

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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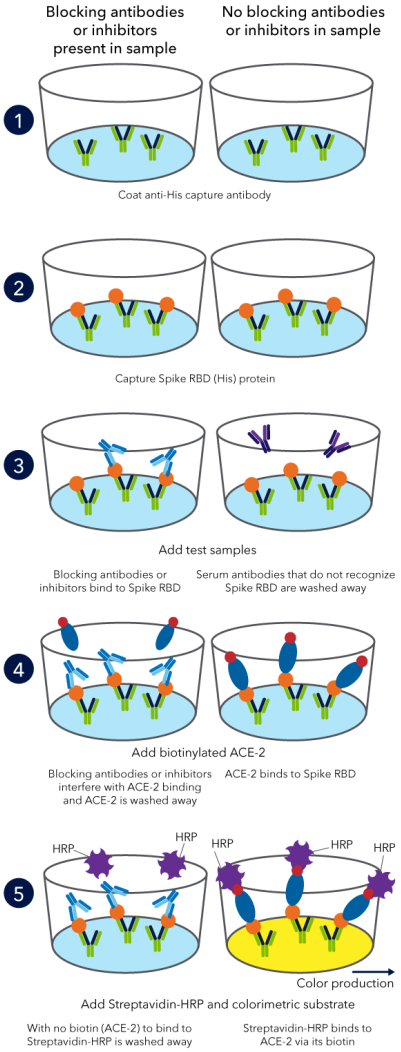
INTRODUCTION

Severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2), the virus that causes COVID-19, has posed a serious threat to global human health. With the emergence of variants, especially the Variants of Concern (VOCs), a quick and effective way to assess how effective the current vaccines are against the major variants is needed. The SARS-CoV-2 Variant Inhibitor Screening Kit is an ELISA type of binding assay measuring the ability of the sera of vaccinated donors to block the binding between RBDs of COVID-19 variants and human ACE-2.

PRINCIPLE OF THE ASSAY

SARS-CoV-2 Variant Inhibitor Screening Kit is a microplate-based binding assay. Recombinant SARS2 Spike RBD His-Tag wild type or variants is immobilized onto His-Tag Capture Antibody coated plate. After pre-incubating immobilized Recombinant SARS2 Spike RBD with serially diluted vaccinated serum or plasma samples or inhibitors/drug targets, Biotinylated Recombinant Human ACE-2 is added to the plate. The binding between immobilized Recombinant SARS2 Spike RBD His-Tag and Biotinylated Recombinant Human ACE-2 is determined by Streptavidin-HRP followed by color reaction. The ability of antibodies generated by vaccines or inhibitors/drug targets inhibiting the binding between RBDs of COVID-19 variants and ACE-2 will be determined by comparing optical density readings among different experimental group.



PRECAUTIONS

The Stop Solution suggested in this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

LIMITATIONS OF PROCEDURES

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

TECHNICAL HINTS

- We recommend the use of R&D Systems® Reagent Diluent Concentrate 2 (Catalog # DY995) to prepare Assay Buffer for use in this assay.
- The use of high quality Bovine Serum Albumin (BSA) for the Assay Buffer is crucial for the optimum performance of the SARS-CoV-2 Variant Inhibitor Screening Kit. Impurities such as proteases, binding proteins, soluble receptors or other interfering substances can be found to varying degrees in virtually all BSA preparations and can cause variation in binding.
- Avoid microbial contamination of reagents and buffers.
- **DO NOT STACK PLATES DURING THE ASSAY INCUBATION STEPS. SPREAD OUT AS A SINGLE LAYER.**
- When mixing or reconstituting solutions, avoid excessive foaming.
- To avoid cross-contamination, change pipette tips between control additions, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Individual results may vary due to differences in technique, plasticware and water sources.
- It is recommended that all samples be assayed in duplicate.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay accuracy.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.
- Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/RECONSTITUTED MATERIAL
His-Tag Capture Antibody	899495	1 vial	May be stored for up to 1 month at 2-8 °C.
rSARS2 Spike RBD Wild Type	899488	1 vial	
rSARS2 B.1.1.7 Spike RBD Alpha Variant*	899489	1 vial	
rSARS2 B.1.351 Spike RBD Beta Variant*	899491	1 vial	
rSARS2 P.1 Spike RBD Gamma Variant*	899492	1 vial	
rSARS2 B.1.617.2 Spike RBD Delta Variant*	899493	1 vial	
rSARS2 B.1.1.529 Spike RBD Omicron Variant*	899518	1 vial	
Biotinylated Human ACE-2	899494	1 vial	
Streptavidin-HRP A	890803	1 vial	
Sample Dilution Plates	N/A	5 plates	

*See Supplement SARS-CoV-2 Variants on page 12.

OTHER MATERIALS AND REAGENTS REQUIRED

- **Tween® 20** (Sigma, Catalog # P2287) or equivalent
- **DuoSet™ Ancillary Reagent Kit 2 (5 plates):**
  (R&D Systems®, Catalog # DY008) containing 96 well microplates, plate sealers, substrate solution, stop solution, plate coating buffer (PBS), wash buffer, and Reagent Diluent Concentrate 2.

The DY008 components listed above may also be purchased separately:

96 well microplates: (R&D Systems, Catalog # DY990)

Plate Sealers: (R&D Systems, Catalog # DY992)

PBS: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2-7.4, 0.2 µm filtered (R&D Systems, Catalog # DY006)

Wash Buffer: 0.05% Tween® 20 in PBS, pH 7.2-7.4 (R&D Systems, Catalog # WA126)

Reagent Diluent: 1% BSA in PBS, pH 7.2-7.4, 0.2 µm filtered (R&D Systems, Catalog # DY995)
Quality of BSA is critical (see Technical Hints)

Substrate Solution: 1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999)

Stop Solution: 2N H_2SO_4 (R&D Systems, Catalog # DY994)

REAGENT PREPARATION

Bring all reagents to room temperature before use. Allow all components to sit for a minimum of 15 minutes with gentle agitation after initial reconstitution. Working dilutions should be prepared and used immediately.

Blocking Buffer Preparation (PBS-1% BSA) - Dilute Reagent Diluent Concentrate 2 from 10X to 1X in deionized or distilled water (e.g., for every 100 mL of Blocking Buffer needed, add 10 mL of Reagent Diluent Concentrate 2 (10X) and 90 mL of distilled or deionized water).

Assay Buffer Preparation (PBS-1% BSA-0.05% Tween®-20) - Dilute Reagent Diluent Concentrate 2 from 10X to 1X in 0.055% Tween-20. (e.g., for every 100 mL of Assay Buffer needed, add 10 mL of Reagent Diluent Concentrate 2 (10X) and 90 mL of 0.055% Tween-20).

Wash Buffer Preparation (0.05% Tween 20 in PBS) - Dilute Wash Buffer Concentrate from 25X to 1X in distilled or deionized water. (e.g., for every 100 mL of Wash Buffer needed, add 4 mL of Concentrate Wash Buffer (25X) and 96 mL of distilled or deionized water).

His-Tag Capture Antibody (200X) - Reconstitute with 300 µL of PBS to produce a 200X solution.

rSARS2 Spike RBD Wild Type (1000X) - Reconstitute with 100 µL of PBS to produce a 1000X solution.

rSARS2 B.1.1.7 Spike RBD Alpha Variant (1000X) - Reconstitute with 100 µL of PBS to produce a 1000X solution.

rSARS2 B.1.351 Spike RBD Beta Variant (1000X) - Reconstitute with 100 µL of PBS to produce a 1000X solution.

rSARS2 P.1 Spike RBD Gamma Variant (1000X) - Reconstitute with 100 µL of PBS to produce a 1000X solution.

rSARS2 B1.617.2 Spike RBD Delta Variant (1000X) - Reconstitute with 100 µL of PBS to produce a 1000X solution.

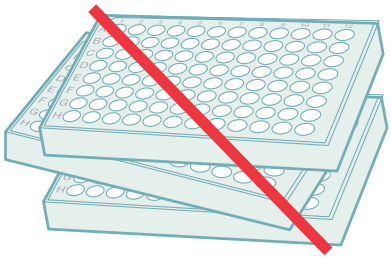
rSARS2 B.1.1.529 Spike RBD Omicron Variant (1000X) - Reconstitute with 100 µL of PBS to produce a 1000X solution.

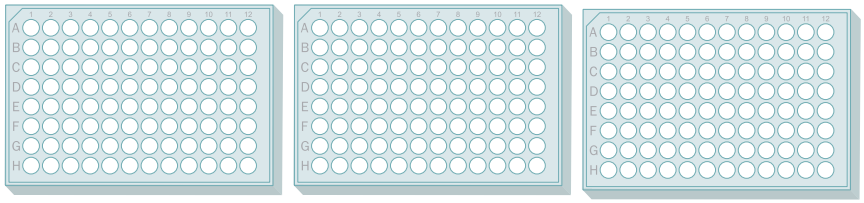
Biotinylated Human ACE-2 (1000X) - Reconstitute with 100 µL of PBS to produce a 1000X solution. **Store at 2-8 °C immediately after reconstitution.**

ASSAY PROCEDURE

Prepare a Plate Layout listing how to coat the desired RBD protein(s) to the Assay Plate and which wells to add each sample. It is suggested to dilute samples in the Sample Dilution Plate and then transfer to the Assay Plate. Plate layouts for the Dilution Plate and Assay Plate are identical.

Note: *The Biotinylated Human ACE-2 (1000X) must remain at 2-8 °C. Bring all buffers and plates to room temperature for a minimum of 15 minutes before use. Do not stack plate for any part of the procedure.*





1. Dilute His-Tag Capture Antibody from 200X to 1X with ELISA Coating Buffer/PBS. Coat the His-Tag Capture Antibody on a 96 well Strip-well Microplates (R&D Systems®, Catalog # DY008) or (R&D Systems, Catalog # DY990), 100 µL/well. **This is the Assay Plate.** (e.g., for every 12 mL of 1X Capture Antibody needed, add 60 µL of His-Tag Capture Antibody (200X) and 11.94 mL of ELISA Coating Buffer/PBS.)
2. Cover with an ELISA plate sealer and incubate overnight at 2-8 °C.
3. The next day, aspirate each well and wash 4 times with 1X Wash Buffer (360 µL/well). Rotate the plate 180 degrees after the second wash. After the last wash, invert plate and blot on clean paper towels.
4. Add 200 µL/well Blocking Buffer and incubate for 60-90 minutes at 37 °C.
5. Following the incubation, remove the Assay Plate from 37 °C and place at room temperature for a minimum of 15 minutes. Aspirate each well and invert plate and blot on clean paper towels.
6. Refer to the Plate Layout to coat the desired RBD protein(s) to the Assay Plate. Prepare a dilution of the desired SARS2 RBD protein from 1000X to 1X in Assay Buffer and add to the plate, 100 µL/well. (e.g., for every 12 mL of 1X SARS2 RBD protein needed, add 12 µL of SARS2 RBD protein from (1000X) and 11.99 mL of Assay Buffer.)

ASSAY PROCEDURE *CONTINUED*

7. Incubate for 60-90 minutes at room temperature.
8. In the **Sample Dilution Plate** (clear 96-well microplate) add 60 µL/well Assay Buffer to all wells of the plate.
9. Refer to the Plate Layout for which wells to add each sample. Add 30 µL of testing sample to the first wells *(For serum or plasma samples, we suggest a 1:9 pre-dilution with Assay Buffer)*. Mix 30 µL/well and make 3-fold serial dilutions, discard the remaining 30 µL/well from the last wells. Do not add testing sample to RBD only and NSB control wells. (e.g., for every 324 µL 1:9 pre-diluted sample needed (or enough to use in duplicate for five RBD proteins), add 36 µL serum or plasma samples to 288 µL of Assay Buffer to produce a final volume of 324 µL 1:9 pre-diluted sample).
10. Aspirate each well of the Assay Plate and wash 4 times with 1X Wash Buffer (360 µL/well). Rotate the plate 180 degrees after the second wash. After the last wash, invert plate and blot on clean paper towels.
11. Mix and transfer 50 µL/well from the Sample Dilution Plate to the Assay Plate. Use new tips for each well. After transferring, discard the Working Plate.
12. Incubate for 60-90 minutes at room temperature.
13. Prepare a dilution of Biotinylated Human ACE-2 from 1000X to 1X in Assay Buffer and add 50 µL/well to all wells of the Assay Plate making the final volume 100 µL/well. Use new tips for each well. (e.g., for every 12 mL of 1X Biotinylated Human ACE-2 needed, add 12 µL of Biotinylated Human ACE-2 (1000X) and 11.99 mL of Assay Buffer.)
    Note: *Keep Biotinylated Human ACE-2 (1000X) on ice while preparing dilutions.*
14. Gently tap the plate to ensure thorough mixing. Incubate for 90 minutes at room temperature.
15. Aspirate each well and wash 4 times with 1X Wash Buffer (360 µL/well). Rotate the plate 180 degrees after the second wash. After the last wash, invert plate and blot on clean paper towels.

ASSAY PROCEDURE *CONTINUED*

16. Prepare a dilution of Streptavidin-HRP Conjugate at a 1:200 dilution in Assay Buffer and add 100 µL/well to all wells. (e.g., for every 12 mL of 1:200 Streptavidin-HRP Conjugate needed, add 60 µL of SA-HRP Conjugate and 11.94 mL of Assay Buffer.)

17. Incubate for 30 minutes at room temperature.

18. Aspirate each well and wash 4 times with Wash Buffer (1X) (360 µL/well). Rotate the plate 180 degrees after the second wash. After the last wash, invert plate and blot on clean paper towels.

19. Add 100 µL of Substrate Solution to each well. Protect from light.

20. Incubate for 15-25 minutes at room temperature.

21. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

22. Determine optical density (OD) of each well within 15 minutes at 450 nm with a correction wavelength at 540 or 570 nm.

23. Calculation: If samples are tested in duplicates, calculate average OD. Use Average OD to calculate % Blocking:

$$\% \text{ Blocking} = [(OD_{\text{RBD only}} - OD_{\text{NSB}}) - (OD_{\text{sample}} - OD_{\text{NSB}})] / (OD_{\text{RBD only}} - OD_{\text{NSB}}) * 100$$

DATA EXAMPLE

Serum sample collected was tested using this kit.

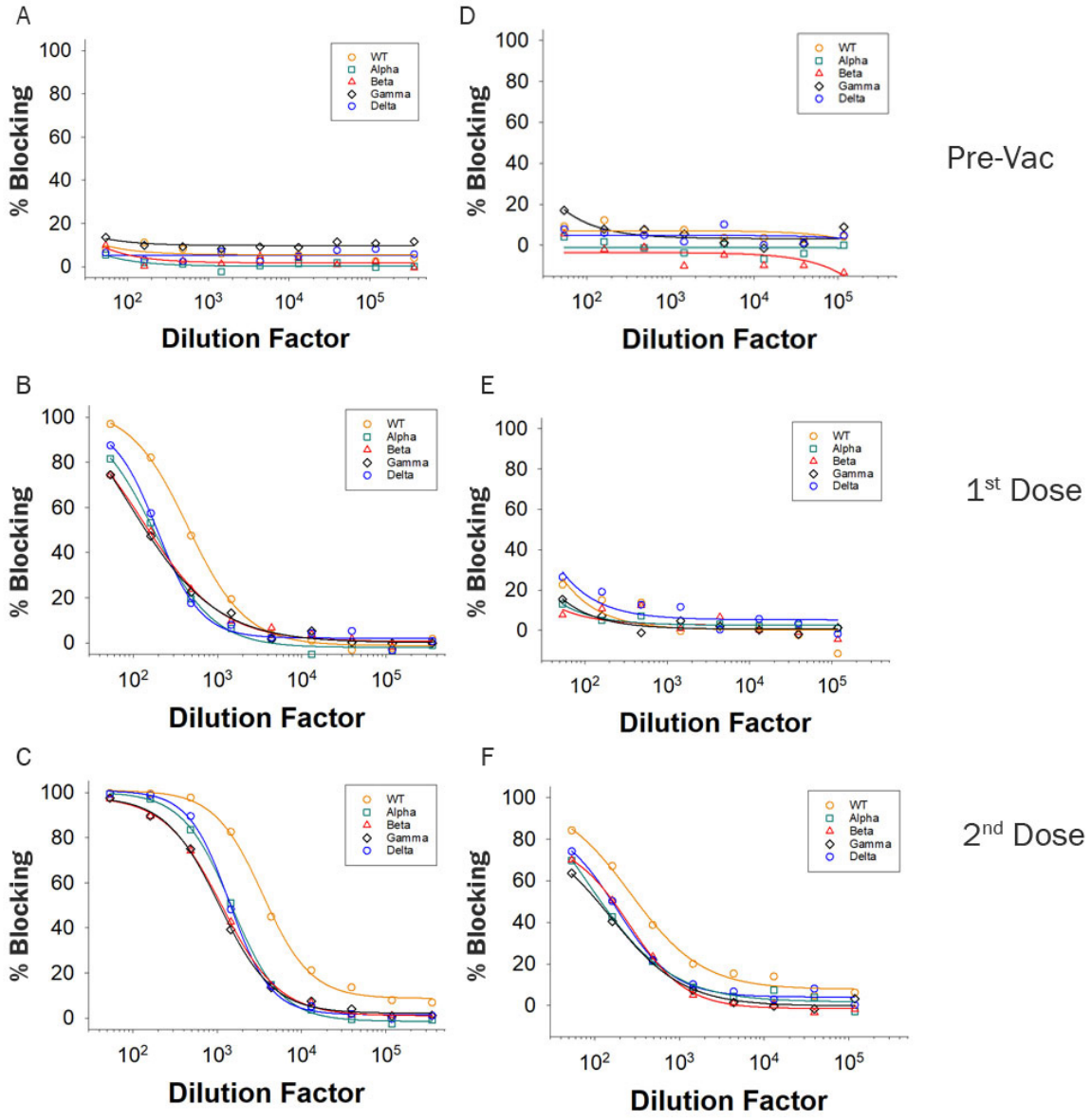


Figure 1: Example data using vaccinated donor samples. Plasma samples were collected from two donors over a time course consisting of Pre-Vaccination (Pre-Vac), or after receiving the 1st Dose or 2nd Dose of the Moderna Vaccine. Donor 1 (A-C) and Donor 2 (D-F) had different blocking antibody levels after the first and second doses. In addition, increasing amounts of blocking antibodies were present at higher samples Dilution Factors after each dose. The differences in blocking efficiency of ACE-2 association between the Wild Type (WT), Alpha, Beta, Gamma, or Delta variant Spike RBD was measured.

DATA EXAMPLE *CONTINUED*

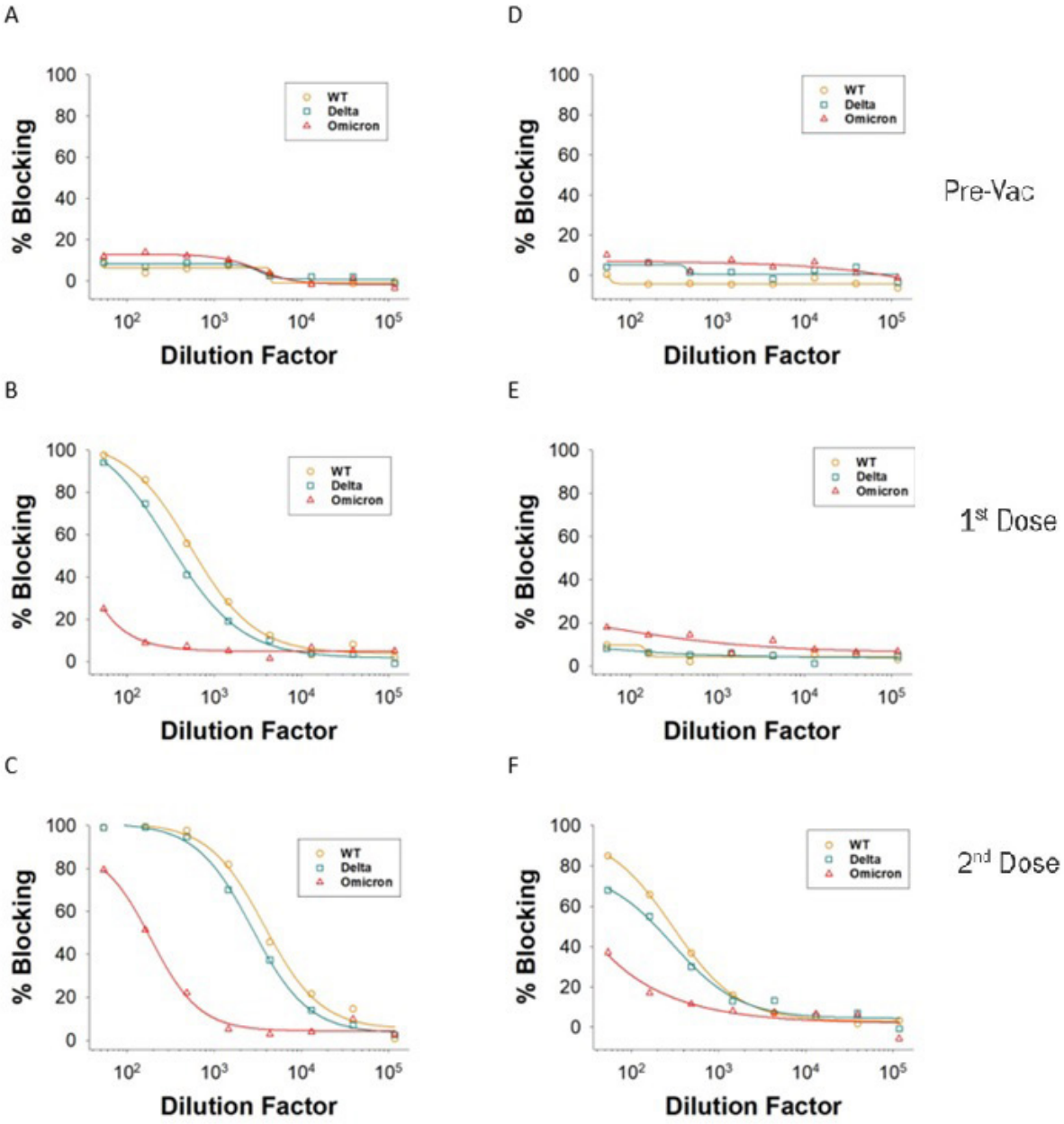


Figure 2: Example data using vaccinated donor samples Using the Wild Type or the Delta and Omicron Variants. Plasma samples were collected from two donors over a time course consisting of Pre-Vaccination (Pre-Vac), or after receiving the 1st Dose or 2nd Dose of the Moderna Vaccine. Donor 1 (A-C) and Donor 2 (D-F) had different blocking antibody levels after the first and second doses. In addition, increasing amounts of blocking antibodies were present at higher samples Dilution Factors after each dose. The differences in blocking efficiency of ACE-2 association between the Wild Type (WT), Delta, or Omicron Variant, Spike RBD was measured.

PLATE LAYOUT A

A suggested plate layout is shown below to screen one testing sample in duplicate in a serial dilution, including controls against the WT Spike RBD and/or five variant Spike RBD proteins for inhibitory activity.

COATED PROTEIN	rSARS2 Spike RBD Wild Type		rSARS2 B.1.1.7 Spike RBD Alpha Variant		rSARS2 B.1.351 Spike RBD Beta Variant		rSARS2 P.1 Spike RBD Gamma Variant		rSARS2 B.1.617.2 Spike RBD Delta Variant		rSARS2 B.1.1.529 Spike RBD Omicron Variant	
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G	Wild Type RBD only control		Alpha RBD only control		Beta RBD only control		Gamma RBD only control		Delta RBD only control		Omicron RBD only control	
H	NSB control											

PLATE LAYOUT B

A suggested plate layout is shown below to screen six testing samples in duplicate in a serial dilution, including controls against a single variant Spike RBD protein (Omicron is shown as an example) for inhibitory activity.

COATED PROTEIN	rSARS2 B.1.1.529 Spike RBD Omicron Variant		rSARS2 B.1.1.529 Spike RBD Omicron Variant		rSARS2 B.1.1.529 Spike RBD Omicron Variant		rSARS2 B.1.1.529 Spike RBD Omicron Variant		rSARS2 B.1.1.529 Spike RBD Omicron Variant		rSARS2 B.1.1.529 Spike RBD Omicron Variant	
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G	Omicron RBD only control		Omicron RBD only control		Omicron RBD only control		Omicron RBD only control		Omicron RBD only control		Omicron RBD only control	
H	NSB control											

APPENDIX

SARS-CoV-2 Variants

WHO LABEL	Pango Linage	Mutations in RBD	GIS/AID clade/ lineage	Nextstrain Clade	Earliest Documented Samples	Date of Designation
ALPHA (α)	B1.1.7	N510Y	GRY (formerly GR/501.Y.V1)	201 (V1)	United Kingdom, September 2020	18/12/2020
BETA (β)	B1.351	K417N,E484K, N501Y	GH/501Y.V2	20H (V2)	South Africa, May 2020	18/12/2020
GAMMA (γ)	P.1	K417T, E484K, N501Y	Gr/501Y.V3	20J (V3)	Brazil, November 2020	11/01/2021
DELTA (δ)	B.1.617.2	L452R, T478K	G/478K.V1	21A	India, October 2020	VOI: 04/04/2021
						VOC: 11/05/2020
OMICRON (ο)	B.1.1.529	G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H	GRA	21K, 21L, 21M	Multiple countries, November 2021	VUM: 24/11/2021
						VOC: 26/11/2021

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