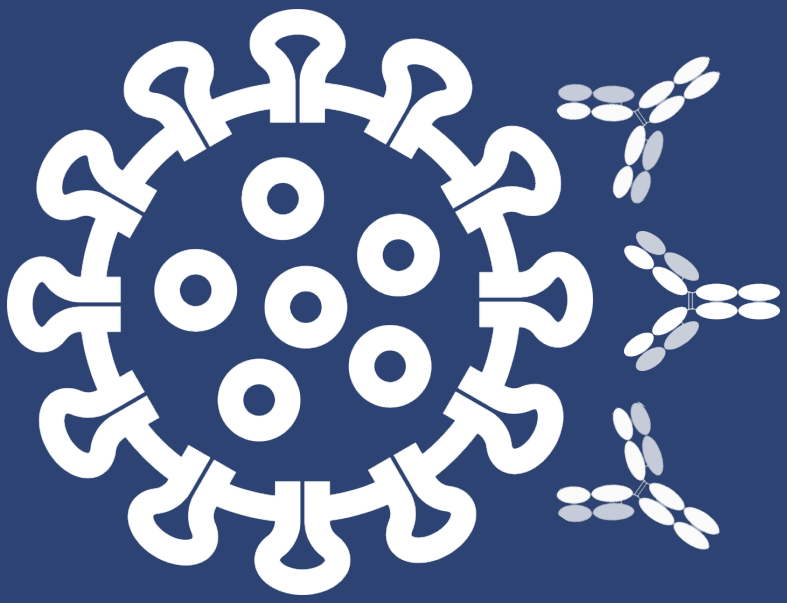

ProteoGenix

Technical Guide and Protocol

SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) Kit

Catalog number KPTX01

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INTRODUCTION

SARS-CoV-2, the etiological agent of the COVID-19 disease, is a pandemic strain of Coronavirus with presumable zoonotic origins. The new strain contains several structural proteins such as the spike glycoprotein (S), envelope (E), membrane (M), and the nucleocapside (N). The strain also contains 16 additional non-structural proteins (Nsp) with different roles in viral infection and replication.

The infection initiates when SARS-CoV-2 binds the human ACE2 receptor (angiotensin-converting enzyme 2) through the receptor-binding domain (RBD) present in the *N*-terminal of the spike glycoprotein. One way to arrest the infectious cycle is to block RBD-ACE2 interaction with neutralizing antibodies.

But due the high virulence of the SARS-CoV-2 strain, viral neutralization tests can only be performed through time-consuming and technically-demanding approaches such as pseudo-virus neutralization assays (pVNT) or in facilities with a strict biosafety containment (BSL3) such as plaque reduction neutralization tests (PRNT).

sVNT TEST PRINCIPLE

Designed in the versatile ELISA format, the sVNT assay allows the quick screening of SARS-CoV-2 neutralizing activity of candidate antibodies without the need to use native viral particles nor mammalian cells. It is compatible with purified antibodies from any species, format, or isotype and it serves to accelerate the development of anti-SARS-CoV-2 antibodies for therapy, diagnostics, and research.

The assay works according to the principle of competitive binding between a purified antibody and horseradish peroxidase labeled ACE2 for the binding pocket of SARS-CoV-2 RBD **[Figure 1]**. Since the label is on the human ACE2, the neutralizing activity of an antibody (Nab) will be inversely proportional to the test signal ($OD_{450\ nm}$).

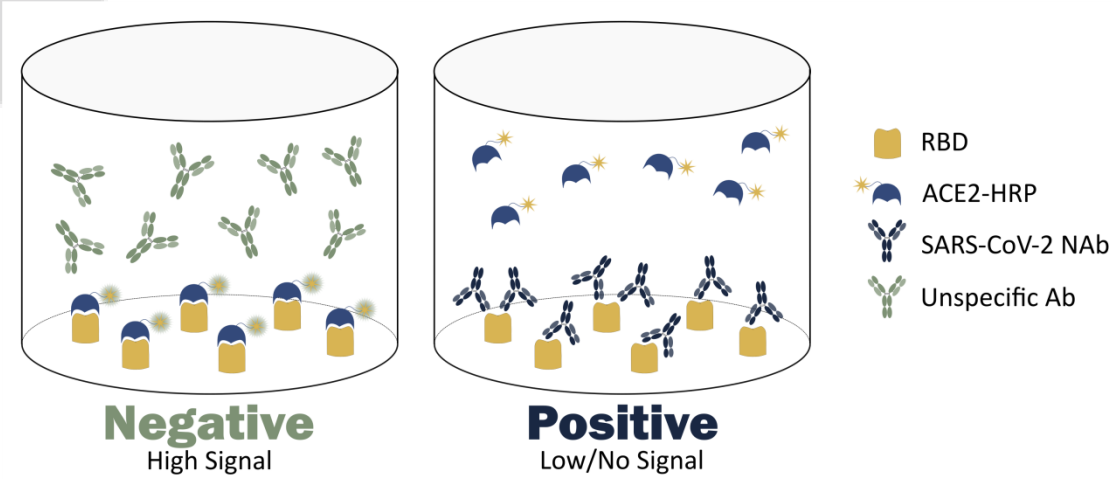

Figure 1. Schematic representation of the **sVNT** principle of detection in ELISA format.

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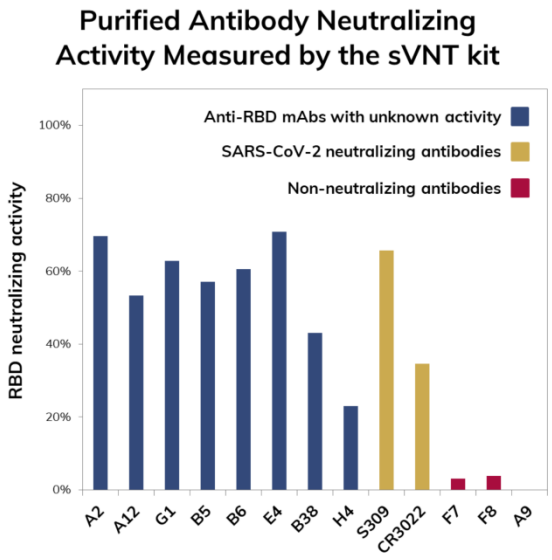
RECOMMENDATIONS FOR PURIFIED ANTIBODIES



These tests were performed with **1 µg/ml** of each purified antibody under the standard protocol.

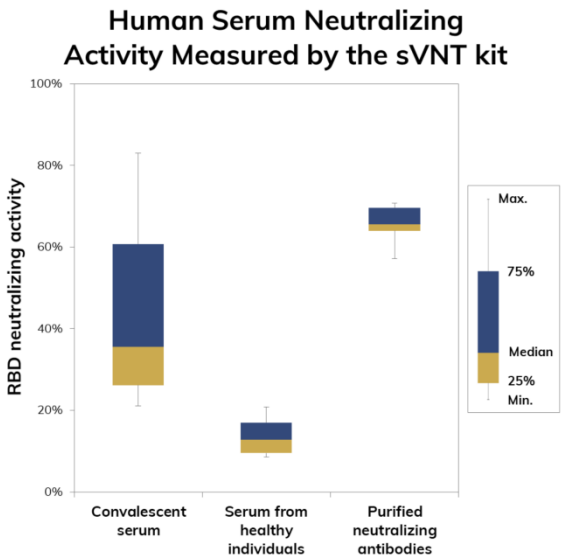
Antibodies **S309** (doi: 10.1038/s41586-020-2349-y) and **CR3022** (doi: 10.1038/s41467-020-16256-y), shown to neutralize SARS-CoV-2 in conventional PRNT assays, were used as positive controls.

Non-neutralizing antibodies were shown to bind to the S2 subunit of SARS-CoV-2 and thus used as **negative controls**.

Anti-RBD antibodies isolated from the **Covid-19 human antibody library** and with confirmed RBD-binding specificity using ELISA and Biacore SPR, served as test samples.

Results indicate that the inhibition ratio (=neutralizing activity) obtained through the sVNT kit correlates strongly with an antibody's potential for neutralizing the SARS-CoV-2 virus.

RECOMMENDATIONS FOR SERUM SAMPLES



Convalescent serum (n = 28), 10-fold diluted, was used to monitor the development of neutralizing activity in patients and compared to samples from healthy individuals and purified antibodies with proven neutralizing activity.

But while a basal signal was detected in the serum from healthy individuals (second box on the boxplot), **the inhibition ratios were significantly inferior** to the ones detected in convalescent serum samples.

These **results are consistent with previous reports** of the variability found in the immune response of different individuals (symptomatic and asymptomatic) to the SARS-CoV-2 virus (doi: 10.1038/s41591-020-0965-6).

Results showed a strong correlation between exposure to SARS-CoV-2 virus and the development of a measurable neutralizing immune response in some patients.

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PRODUCT COMPONENTS

Each sVNT assay kit includes the following components:

COMPONENT	QUANTITY/SIZE
Capture Plate	1 plate (96 wells)
Positive Control	30 µl (1 vial)
Negative Control	30 µl (1 vial)
Biotin Conjugated ACE2	20 µl (1 vial)
Streptavidin-HRP	150 µl (1 vial)
Reagent Dilution Buffer	25 ml (1 vial)
Sample Dilution Buffer	25 ml (1 vial)
20x Wash Solution	25 ml (1 vial)
TMB Solution	12 ml (1 vial)
Stop Solution	6 ml (1 vial)
Plate Sealer	4 pieces

PRODUCT DETAILS

Kit Size	96 tests
Sample Type	Purified Antibody
Instrument	Colorimetric Microplate Reader
Measurements	$OD_{450\ nm}$
Shipping Conditions	Blue Ice
Storage Conditions	Stable for 6 months from the date of manufacture if Capture Plate, Controls, Biotin conjugated ACE2, and Streptavidin-HRP are kept at -20°C. The remaining reagents can be stored at 4°C.
Assay Kit Format	Competitive ELISA
Assay Type	Qualitative
Label or Dye	Horseradish Peroxidase (HRP)
Substrate	3,3′,5,5′-Tetramethylbenzidine (TMB)
Validation data	Tested with purified antibodies (neutralizing and non-neutralizing) and convalescent/healthy human serum
Expected Control Results	Positive Control $OD_{450\ nm} < 0.3$ Negative Control $OD_{450nm} > 1.0$

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TEST PREPARATION

REAGENT PREPARATION

1. Take all reagents from cold storage (-20 or 4 °C) and allow them to return to room temperature before use (20 to 25°C). Store all reagents at the recommended storage temperature promptly after use.
2. Vortex all reagents before use.
3. Biotin-ACE2 Reagent preparation procedure: dilute **Biotin-ACE2** in **Reagent Dilution Buffer** in a 1:600 volume ratio. *Example: to prepare 6 ml of the solution dilute 10 µl of the Biotin conjugated ACE2 in 5.99 ml of Reagent Dilution Buffer.*
4. Streptavidin-HRP Reagent preparation procedure: dilute **Streptavidin-HRP** in **Reagent Dilution Buffer** in a 1:100 volume ratio. *Example: to prepare 10 ml of the solution dilute 100 µl of the Streptavidin-HRP in 9.9 ml of Reagent Dilution Buffer.*
5. 1× Wash Solution preparation procedure: dilute the **20× Wash Solution** in deionized or distilled water in a 1:20 volume ratio. Store the solution at 2-8°C when not in use. *Example: to prepare 400 ml of this solution dilute 20 ml of 20× Wash Solution in 380 ml deionized or distilled water.*

SAMPLE AND CONTROL PREPARATION

6. Take all samples and controls from cold storage (-20 or 4 °C) and allow them to return to room temperature before use (20 to 25°C). Store them back at the recommended storage temperature promptly after use.
7. Vortex all samples and controls before use.
8. Positive and Negative Control preparation procedure: dilute control with the **Sample Dilution Buffer** in a 1:50 volume ratio. *Example: to prepare 500 µl of the control solution dilute 10 µl of each control in 490 µl of Sample Dilution Buffer.*
9. Sample preparation procedure: dilute samples with the **Sample Dilution Buffer** in a 1:10 volume ratio (this may need to be optimized according to the concentration of your sample). *Example: to prepare 100 µl dilute 10 µl of each sample in 90 µl Sample Dilution Buffer.*

CAPTURE PLATE PREPARATION

For more accurate results it is recommended to measure each sample and control in duplicate.

10. Prepare the number of strips according to the number of samples you will be measuring. Install the strips making sure they are tightly snapped to the plate frame.
11. Keep the unused strips in the closed foil pouch (to prevent damage from moisture) and store them at -20 °C.

PROTOCOL

NEUTRALIZATION REACTION

1. In separate tubes, mix the 50-fold diluted **Positive Control**, 50-fold diluted **Negative Control**, and the **Samples** with the diluted **Biotin-ACE2 solution** in a 1:1 volume ratio. *Example: to prepare 120 µl (enough for 1 well) mix 60 µl Sample or Control with 60 µl Biotin-ACE2 solution.*
2. Transfer 100 µl of the pre-mixed Sample or Control to the corresponding wells.
3. Cover the **Capture Plate** with the **Plate Sealer** and incubate at **37 °C for 60 minutes**.
4. Remove the **Plate Sealer** and wash the plate with 300 µl **1× Wash Solution.**
5. Repeat 3 times step (4).
6. Add 100 µl of the diluted **Streptavidin-HRP solution** to each well.
7. Cover the plate with **Plate Sealer** and incubate at **37 °C for 30 additional minutes**.
8. Remove the **Plate Sealer** and wash the plate with 300 µl **1× Wash Solution**.
9. Repeat 5 times step (8).
10. Pat the plate on a clean paper towel to remove residual liquid in the wells after washing.

SUBSTRATE REACTION AND MEASUREMENT

11. Add 100 µl of **TMB Solution** to each well and incubate the plate in the dark at **37 °C for 5 minutes** (start timing after the addition of TMB Solution to the first well).
12. Add 50 µl of **Stop Solution** to each well to quench the reaction.
13. Read the absorbance in a microplate reader at 450 nm immediately after stopping the reaction.

*Note: Substrate reaction time is determined by the reaction temperature. The ideal reaction temperature is **37 °C**. If the temperature is lower than 37 °C, extend the reaction time appropriately.*

TEST QUALITY CONTROL

POSITIVE CONTROL	$OD_{450\ nm} < 0.3$
NEGATIVE CONTROL	$OD_{450\ nm} > 1.0$

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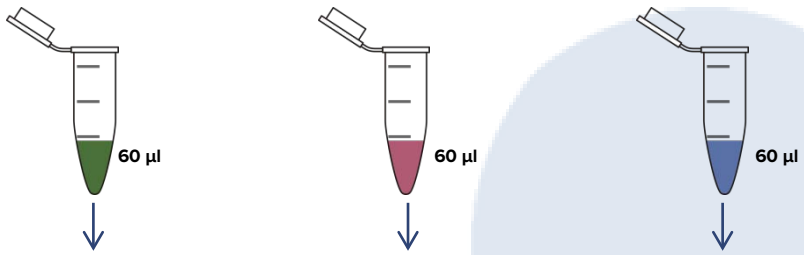
SIMPLIFIED PROTOCOL

Add 60 µl **Biotin-ACE2** (600-fold diluted)

Positive control | **Negative control** | **Sample**



Transfer 100 µl of each to a well in the **Capture Plate**

60

Seal the plate and incubate at **37 °C for 60 min**

Wash 3x with 300 µl **1x Wash Solution**

Add 100 µl **Streptavidin-HRP** (100-fold diluted) to each well

30

Seal the plate and incubate at **37 °C for 30 min**

Wash 5x with 300 µl of **1x Wash Solution**

5

Add 100 µl **TMB** to each well and incubate for **5 min at 37 °C**

Add 50 µl of **Stop Solution** and read absorbance at **450 nm**