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SARS-CoV-2 Surrogate Virus Neutralization Test Kit

For Research Use Only. Not for Use in Diagnostic Procedures.

The operator should read this technical manual carefully before using this product.

Contents

I. INTENDED USE

The GenScript SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) Kit can detect circulating neutralizing antibodies against SARS-CoV-2 that block the interaction between the receptor binding domain of the viral spike glycoprotein (RBD) with the ACE2 cell surface receptor. The assay detects any antibodies in serum and plasma that neutralize the RBD-ACE2 interaction. The test is both species and isotype independent.

II. BACKGROUND

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, or 2019-nCoV) is an enveloped non-segmented positive-sense RNA virus. It is the cause of coronavirus disease 2019 (COVID-19), which is contagious in humans.

SARS-CoV-2 has several structural proteins including spike (S), envelope (E), membrane (M) and nucleocapsid (N). The spike protein (S) contains a receptor binding domain (RBD), which is responsible for recognizing the cell surface receptor, angiotensin converting enzyme-2 (ACE2). It is found that the RBD of the SARS-CoV-2 S protein strongly interacts with the human ACE2 receptor leading to endocytosis into the host cells of the deep lung and viral replication.

Infection with the SARS-CoV-2 initiates an immune response, which includes the production of antibodies in the blood. The secreted antibodies provide protection against future infections from viruses, because they remain in the circulatory system for months to years after infection and will bind quickly and strongly to the pathogen to block cellular infiltration and replication. These antibodies are named neutralizing antibodies.

III. ASSAY PRINCIPLE

The SARS-CoV-2 sVNT Kit is a blocking ELISA detection tool, which mimics the virus neutralization process. The kit contains two key components: the Horseradish peroxidase (HRP) conjugated recombinant SARS-CoV-2 RBD fragment (HRP-RBD) and the human ACE2 receptor protein (hACE2). The protein-protein interaction between HRP-RBD and hACE2 can be blocked by neutralizing antibodies against SARS-CoV-2 RBD.

First, the samples and controls are pre-incubated with the HRP-RBD to allow the binding of the circulating neutralization antibodies to HRP-RBD. The mixture is then added to the capture plate which is pre-coated with the hACE2 protein. The unbound HRP-RBD as well as any HRP-RBD bound to non-neutralizing antibody will be captured on the plate, while the circulating neutralization antibodies_HRP-RBD complexes remain in the supernatant and get removed during washing. After washing steps, TMB solution is added, making the color blue. By adding Stop Solution, the reaction is quenched and the color turns yellow. This final solution can be read at 450 nm in a microtiter plate reader. The absorbance of the sample is inversely dependent on the titer of the anti-SARS-CoV-2 neutralizing antibodies.

IV. KIT CONTENTS

Component	96 Tests		480 Tests	
	Quantity	Part No.	Quantity	Part No.
Capture Plate	1 plate	S1-80	5 plates	S5-80
Positive Control	1 vial (0.05 mL)	S1-10	1 vial (0.25 mL)	S5-10
Negative Control	1 vial (0.05 mL)	S1-11	1 vial (0.25 mL)	S5-11
HRP conjugated RBD	1 vial (0.02 mL)	S1-30	1 vial (0.1 mL)	S5-30
HRP Dilution Buffer	1 bottle (10 mL)	S1-90	1 bottle (50 mL)	S5-90
Sample Dilution Buffer	1 bottle (30 mL)	S1-60	2 bottles (150 mL)	S5-60
20× Wash Solution	1 bottle (40 mL)	S1-70	2 bottles (200 mL)	S5-70
TMB Solution	1 bottle (12 mL)	S1-40	1 bottle (60 mL)	S5-40
Stop Solution	1 bottle (6 mL)	S1-50	1 bottle (30 mL)	S5-50
Plate Sealer	2 pieces	N/A	10 pieces	N/A

- Capture Plate: 96 well microplates (8 wells x 12 strips) pre-coated with recombinant ACE2 protein; 12 strips configured in plate; Plate sealed in a foil pouch with a desiccant.

V. STORAGE

The unopened kit is stable for at least 12 months from the date of manufacture if stored at 2°C to 8°C, and the opened kit is stable for up to 1 month from the date of opening at 2°C to 8°C.

VI. REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

- Single or dual wavelength microplate reader with 450 nm filter. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance

specifications of the reader.

- Automated microplate washer to wash the plate
- Deionized or distilled water to dilute 20× Wash Solution
- Graduated cylinder to prepare Wash Solution
- Plastic container to store Wash Solution
- Tubes to aliquot and dilute samples
- 10 µL, 200 µL and 1000 µL precision pipettes
- 10 µL, 200 µL and 1000 µL pipette tips
- Multichannel pipettes
- Disposable reagent reservoir
- Paper towels
- Laboratory timer
- Refrigerator to store samples and kit components
- Centrifuge
- 37 ºC Incubator

VII. PRECAUTIONS

1. Although this product itself does not contain any materials or reagents that can cause infection, the blood or sera collected from SARS-CoV-2 patients (whether newly infected or recovered), and “uninfected” people (could still have potential infectious agents) must be handled using high level of precaution.
2. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents should be handled at the Biosafety Level 2 facility.
3. Do not mix components from different batches. Do not mix with components from other manufacturers.
4. Do not use reagents beyond the stated expiration date.
5. All reagents must be allowed to equilibrate to room temperature (20° to 25°C) before running assay. Remove only the volume of reagents that is needed. Do not pour

reagents back into vials as reagent contamination may occur.

6. Before opening the Positive and Negative Controls, tap the vials on the benchtop to ensure that all liquid is at the bottom of the vial.
7. Use only distilled or deionized water and clean glassware.
8. Do not let wells dry during test, add reagents immediately after completing washing steps.

VIII. SPECIMEN COLLECTION AND STORAGE

1. Handle all blood and serum as if capable of transmitting infectious agents.
2. The NCCLS provides recommendations for handling and storing serum and plasma specimens (Approved Standard-Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).
3. For performance of the GenScript SARS-CoV-2 sVNT, a minimum volume of 50 µL per serum or plasma sample is recommended, in case that repeat testing is required. Specimens should be collected aseptically by venipuncture. Early separation from the clot prevents hemolysis of serum.
4. For human/animal serum, use a blood separator tube and allow sample to clot for 30 minutes, then centrifuge for 10 minutes at 1000 g. Run assay immediately, otherwise store aliquot below -20°C. Avoid repeated freeze-thaw cycles.
5. For human/animal plasma, treat blood with anticoagulant such as citrate, EDTA or heparin. Centrifuge for 10 minutes at 1000 g within 30 minutes for plasma collection. Run assay immediately, otherwise store samples below -20°C. Avoid repeated freeze-thaw cycles.

IX. PROTOCOL

■ Reagent Preparation

1. All reagents must be taken out from refrigeration and allowed to return to room temperature before use (20° to 25°C). Save all reagents in refrigerator promptly after use.

2. All samples and controls should be vortexed before use.
3. HRP-RBD Preparation: Dilute HRP conjugated RBD with HRP Dilution Buffer with a volume ratio of 1:1000. For example, for one 96 well plate testing, dilute 10 μL of HRP conjugated RBD with 10 mL of HRP Dilution Buffer to make a HRP-RBD working solution.
4. 1× Wash Solution Preparation: Dilute the 20× Wash Solution with deionized or distilled water with a volume ratio of 1:19. For example, dilute 40 mL of 20× Wash Solution with 760 mL of deionized or distilled water to make 800 mL of 1× Wash Solution. Store the solution at 2°C to 8°C when not in use.

Note: If any precipitate is observed in the 20× Wash Solution, incubate the bottle in a water bath (up to 50°C) with occasionally mixing until all the precipitate is dissolved.

■ **Sample and Control Dilution**

Dilute test samples, Positive, and Negative Controls with Sample Dilution Buffer with a volume ratio of 1:9. For example, dilute 10 μL of sample with 90 μL of Sample Dilution Buffer.

■ **Capture Plate Preparation**

1. It is recommended that all Positive Control and Negative Control should be prepared in duplicate.
2. Count the strips according to the number of test samples and install the strips. Make sure the strips are tightly snapped into the plate frame.

Test Configuration

	1	2	3	4	5	6	7	8	9	10	11	12
A	Negative Control											
B	Negative Control											
C	Positive Control											
D	Positive Control											
E												
F												
G												
H												

3. Leave the unused strips in the foil pouch and store at 2°C to 8°C. The strips must be

stored in the closed foil pouch to prevent moisture from damaging the Capture Plate.

■ Test Procedure

Neutralization Reaction

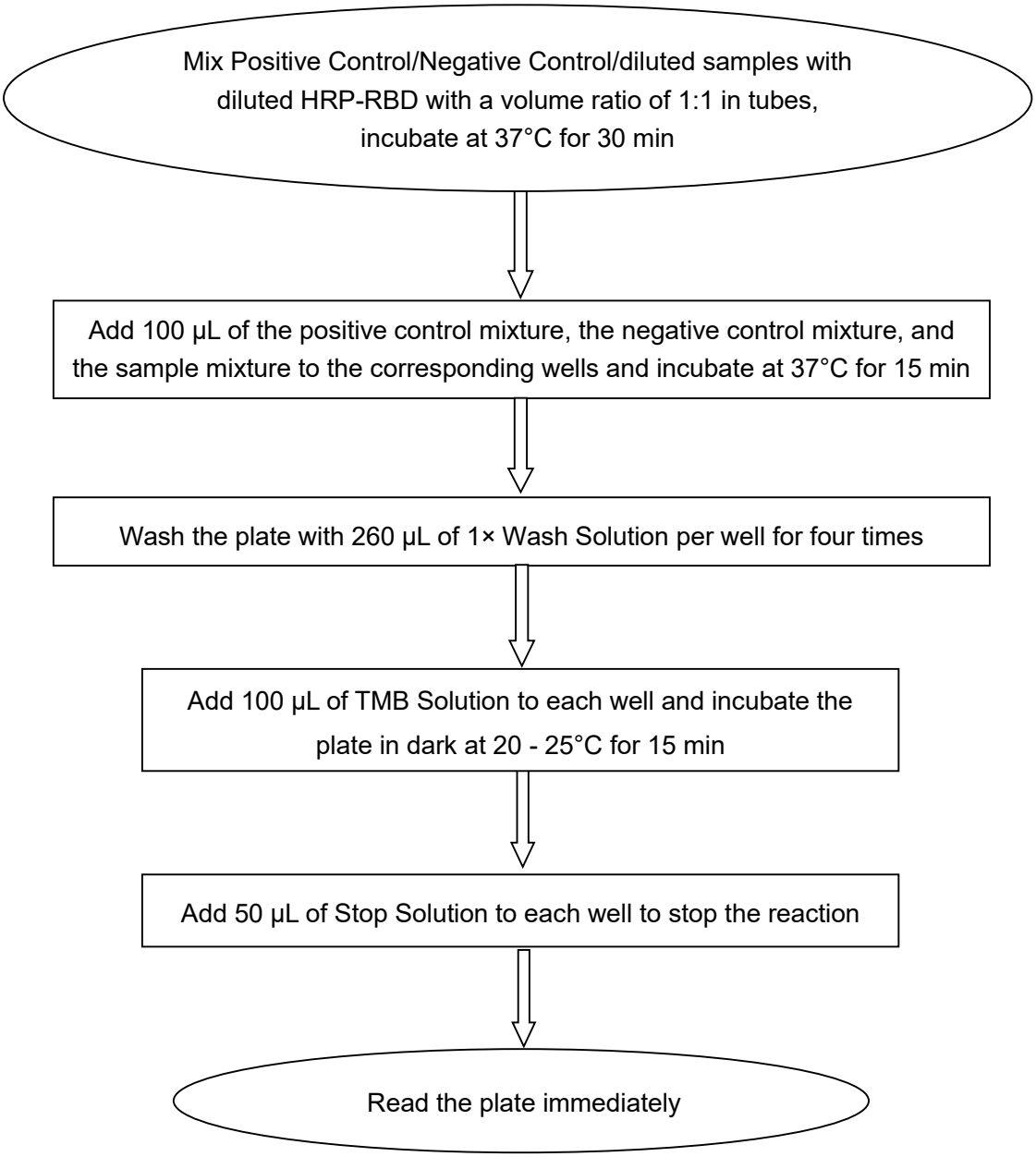
1. In separate tubes, mix the diluted Positive Control, diluted Negative Control, and the samples with the diluted HRP-RBD solution with a volume ratio of 1:1. For example, mix 60 µL Positive Control with 60 µL HRP-RBD solution. Incubate the mixtures at 37°C for 30 minutes.
2. Add 100 µL each of the positive control mixture, the negative control mixture, and the sample mixture to the corresponding wells.
3. Cover the plate with Plate Sealer and incubate at 37°C for 15 minutes.
4. Remove the Plate Sealer and wash the plate with 260 µL of 1× Wash Solution for four times.
5. Pat the plate on paper towel to remove residual liquid in the wells after washing steps.

Substrate Reaction and Absorbance Measurement

6. Add 100 µL of TMB Solution to each well and incubate the plate in the dark at 20 - 25°C for 15 minutes (start timing after the addition of TMB Solution to the first well).
7. Add 50 µL of Stop Solution to each well to quench the reaction.
8. Read the absorbance in the microtiter plate reader at 450 nm immediately.

Note: The substrate reaction time is determined by the temperature, the ideal reaction temperature is 25℃. If the temperature is lower than 25℃, extend the reaction time appropriately.

X. ASSAY PROCEDURE SUMMARY



XI. QUALITY CONTROL

To assure the validity of the results, each assay must include both Positive and Negative Controls. The net optical density (OD450) of each control must fall within the ranges listed in the following table. If OD450 values of controls do not meet the requirements in the following table, the test is invalid and must be repeated.

- OD450 values for quality control

Items	OD450 value	Control Result for Valid Assay
Quality Control	＞ 1.0	Negative Control
	＜ 0.3	Positive Control

Note：The standards in the table are only intended to evaluate the performance of the kit.

XII. INTERPRETATION OF RESULTS

The positive cutoff and negative cutoff for SARS-CoV-2 neutralizing antibody detection can be used for interpretation of the inhibition rate. The operator can determine the result of the sample by comparing the inhibition rate to the following table.

$$\text{Inhibition} = \left(1 - \frac{\text{OD value of Sample}}{\text{OD value of Negative Control}}\right) \times 100\%$$

- Cutoff Interpretation*

Items	Cutoff	Result	Interpretation
SARS-CoV-2 neutralizing antibody test	⩾ 30%	Positive	SARS-CoV-2 neutralizing antibody detected
	＜ 30%	Negative	No detectable SARS-CoV-2 neutralizing antibody

*The cutoff value is based on validation with our panel of confirmed COVID-19 patient sera and healthy control sera. Users may want to set up their own cutoff based on different patient serum panels from different geographic locations or different ethnic backgrounds.

XIII. LIMITATIONS OF THE PROCEDURE

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1. This test is designed for qualitative detection.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the manual is necessary to obtain reliable test results.

3. A negative result can occur if the titer of antibodies against the SARS-CoV-2 virus present in the specimen is below the sensitivity of the kit.
4. If symptoms persist and the result from the SARS-CoV-2 neutralization test is negative, it is recommended to collect a new sample from the patient a few days later and test it again.

XIV. PRECISION

- Intra-assay: One known level of control was spiked into sample buffer as a test sample. The sample was tested 10 times on the same plate to evaluate intra-assay precision of the kit. Intra-assay variation of this kit is less than or equal to 10%.
- Inter-assay: One known level of control was spiked into sample buffer as a test sample. The sample was tested on 3 plates which were randomly selected from 3 different lots to evaluate inter-assay precision of the kit. Inter-assay variation of this kit is less than or equal to 15%.

XV. CLINICAL PERFORMANCE

In order to validate the clinical performance of the GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit, the comparator Plaque Reduction Neutralization Test (PRNT) utilizing the SARS-CoV-2 virus (WA01/2020 isolate) was used. The cutoff for the PRNT comparator tests was established as indicated below:

$PRNT_{50}$:

Value Result (dilution titer)	Result	Test Result Interpretation
⩾ 1:20	Positive	Neutralizing antibodies for SARS-CoV-2 are detected at 50% viral neutralization.
⩽ 1:20	Negative	Neutralizing antibodies for SARS-CoV-2 are not detected at 50% viral neutralization.

$PRNT_{90}$:

Value Result (dilution titer)	Result	Test Result Interpretation
⩾ 1:10	Positive	Neutralizing antibodies for SARS-CoV-2 are detected at 90% viral neutralization.
⩽ 1:10	Negative	Neutralizing antibodies for SARS-CoV-2 are not detected at 90% viral neutralization.

The clinical agreement study evaluated a total of 114 samples retrospectively collected from SARS-CoV-2 RT-PCR positive and negative individuals (26 PRNT positive and 88 PRNT negative) using the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit and the PRNT comparator ($PRNT_{50}$ and $PRNT_{90}$). The combined cohort consisted of samples from normal healthy people (n=88) and samples from RT-PCR confirmed SARS-CoV-2 positive patients (n=26). The GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit sample results were compared to a Plaque Reduction Neutralization Test performed to WHO guidelines. The following tables show the Positive and Negative Percent Agreement between the $PRNT_{50}$ or $PRNT_{90}$ and the cPass SARS-CoV-2 Neutralization Antibody Detection Kit result.

Clinical Agreement using $PRNT_{50}$ titers as the comparator method

		Plaque Reduction Neutralization Test ($PRNT_{50}$)	
		Positive (n=26)	Negative (n=88)
GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit	Positive	26	0
	Negative	0	88
	Positive Percent Agreement	100% (95% CI 87.1-100.0%)	
	Negative Percent Agreement		100.0% (95% CI 95.8-100.0%)

Clinical Agreement using $PRNT_{90}$ titers as the comparator method

		Plaque Reduction Neutralization Test ($PRNT_{90}$)	
		Positive (n=26)	Negative (n=88)
GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit	Positive	26	0
	Negative	0	88
	Positive Percent Agreement	100% (95% CI 87.1-100.0%)	
	Negative Percent Agreement		100.0% (95% CI 95.8-100.0%)

XVI. REFERENCES

1. Chinese Center for Disease Control and Prevention (2020) Public protection guidelines for Novel coronavirus pneumonia, People's Medical Publishing House (PMPH).
2. ZHOU Peng, YANG Xinglou. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature, 2020.
3. XUE Xiongyan, ZHU Changlin, HUANG Shaozhen, Inactivation of 2019 new coronary virus before antibodies detection by different methods. Journal of Southern Medical University, 2020.
4. SHI Heshui, HAN Xiaoyu, FAN Yanqing. Radiologic Features of Patients with 2019-n Co V Infection. Journal of Clinical Radiology, 2020.
5. NCCLS. 1991. National Committee for Clinical Laboratory Standard. Internal Quality
6. Testing of Reagent Water in the Clinical Laboratory. NCCLS Publication C3-A3.
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XVII. TROUBLESHOOTING

Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Wells are scratched with pipette tip or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates are found in the samples	Remove any particulates by centrifugation prior to the assay
Weak/No Signal	Substrate is not added or added at the wrong time	Follow the manual to add the substrate properly
	Components are used from other lots or sources	Use only lot-specific components
	Substrate is contaminated	Use new Substrate with same Lot
	Volumes of reagents are not correct	Repeat assay with the required volumes in manual
	The plate is not incubated for proper time or temperature	Follow the manual to repeat assay
	The plate is not read within the specified time range	Read the plate within 5 minutes
High Background	Plate is not washed properly	Make sure the wash apparatus works properly
	Substrate is contaminated	Use new substrate with same Lot
	Evaporation of wells during incubations	Perform incubation steps with plate sealer in repeat assay
	Incorrect incubation times and/or temperatures	Follow the manual to repeat the assay