cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit

Instruction for Use

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

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

Contents

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

I. PRODUCT NAME

cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit

SARS-CoV-2 Surrogate Virus Neutralization Test Kit

II. PACKING SPECIFICATION

96 Tests/Kit, 480 Tests/Kit

III. INTENDED USE

The GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit can detect all types of neutralizing antibodies against SARS-CoV-2 in serum and plasma, in a species- and isotype-independent manner. The GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating a prior infection. The GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit should not be used to diagnose acute SARS-CoV-2 infection.

IV. 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 causative agent 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

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

future infections from viruses, because they remain in the circulatory system for months to years after infection. A subpopulation of the circulating antibodies can block cellular infiltration and replication of the virus. These antibodies are named neutralizing antibodies.

V. PRINCIPLE

The cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit is a blocking ELISA detection tool. Using purified receptor binding domain (RBD) protein from the viral spike (S) protein and the host cell receptor ACE2, this test is designed to mimic the virus-host interaction by direct protein-protein interaction in a test tube or a well of an ELISA plate. The highly specific interaction can then be neutralized, the same manner as in a conventional Virus Neutralization Test (VNT).

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.

Samples and controls diluted with sample dilution buffer 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. Following a wash cycle, TMB substrate solution is added followed by the Stop Solution, the reaction is quenched and the color turns yellow. The absorbance of the final solution can be read at 450 nm in a microtiter plate reader. The absorbance of the sample is inversely dependents on the titer of the anti-SARS-CoV-2 neutralizing antibodies.

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

VI. KIT CONTENTS

Component	96 Tests		480 Tests	
	Quantity	REF	Quantity	REF
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: Pre-coated 96 well microplates (8 wells x 12 strips); 12 strips configured in plate sealed in a foil pouch with a desiccant.

VII. STORAGE CONDITION AND EXPIRE DATE

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

VIII. 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.

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

- 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 towel
- Laboratory timer
- Refrigerator to store samples and kit components
- Centrifuge
- 37ºC Incubator

IX. PRECAUTIONS

1. For *in vitro* diagnostic use.
2. This product requires the handling of human specimen. It is recommended that all human – sourced materials and all consumables contaminated with potentially infectious materials will be considered potentially infectious and handled in accordance with standard precaution for infection control.
3. Operators should be professionally trained and have experience.
4. Do not use the kit if there is any visible damage or deviation in physical appearances of components as stated under Section VI. KIT CONTENTS.
5. Do not mix components from different batches. Do not mix with components from other manufacturers.
6. Do not use reagents beyond the stated expiration date.
7. All reagents must be at room temperature (20 to 25°C) before running assay. Remove only the volume of reagents that are needed. Do not pour reagents back into vials as reagent contamination may occur.
8. Before opening the Positive and Negative Controls, tap the vial or quick spin to ensure that all liquid is at the bottom of the vial.

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

9. Use only distilled or deionized water and clean glassware.
10. Do not let wells dry during test: add reagents immediately after completing washing steps.
11. Decontaminate and dispose of all specimens, controls, reagents, and other potentially contaminated materials in accordance with local, state, and federal regulations.
12. All materials should be handled in a manner that minimizes the chance of potential contamination of the work area.

X. 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. No prior special preparation is required before sample collection by approved techniques. Collect the specimen in accordance with normal laboratory practice. Specimens should be collected aseptically by venipuncture. Early separation from the clot prevents hemolysis of serum.
4. Do not use haemolysed, clotted, contaminated and viscous specimen. Specimen containing particulate matter should be centrifuged.
5. Store specimens at -20°C or lower if not tested immediately. Avoid repeated freeze-thaw cycles.
6. The handling and storage information provided here is based on references maintained by the manufacturer. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria to meet specific needs.

XI. TEST METHOD

▪ Reagent Preparation

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

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

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, dilute 10 μL of HRP conjugated RBD with 10 mL of HRP Dilution Buffer to make 10 mL of HRP-RBD 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 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 occasional mixing until all the precipitate is dissolved.

▪ Sample and Control Dilution

Dilute test samples, Positive Control, and Negative Control 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 Controls and Negative Controls should be prepared in duplicate.
2. Count the strips for the assay according to the test configuration and 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 to 8°C. The strips must be

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

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, the diluted Negative Control, and the diluted 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. Tap the plate on paper towel to remove residual liquid in the wells after washing steps.

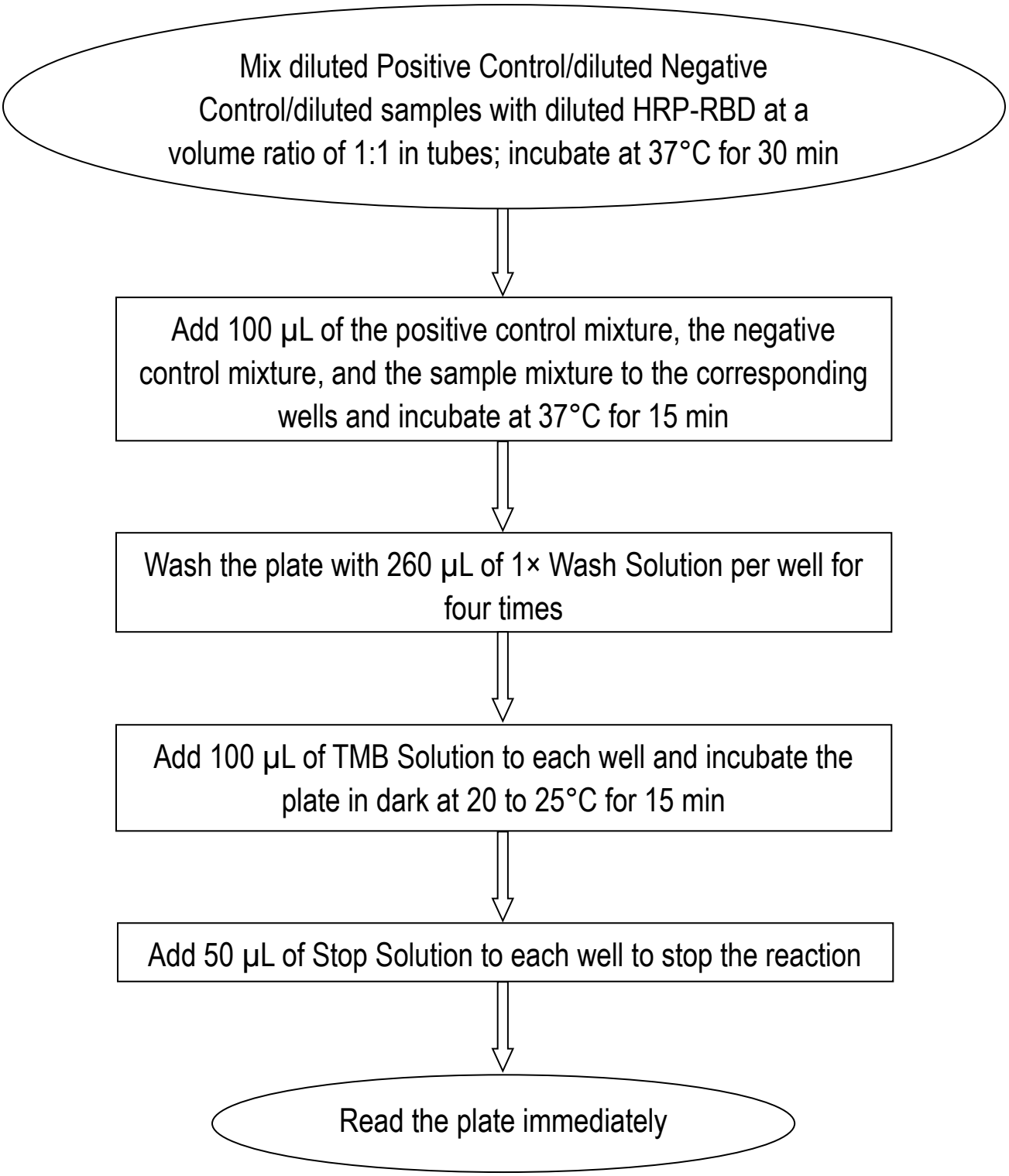
Substrate Reaction and Absorbance Measurement

6. Add 100 µL of TMB Solution to each well and incubate the plate in dark at 20 to 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 microtiter plate reader at 450 nm immediately.

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

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

XII. ASSAY PROCEDURE SUMMARY



Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

XIII. QUALITY CONTROL

To assure the validity of the results, each assay must include both Positive and Negative controls. The average optical density (OD450) of each control must fall within the values 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.

XIV. INTERPRETION OF RESULTS

The average optical density (OD) of the negative control will be used to calculate the inhibition%. Results of each individual samples can be calculated using the formulation as below:

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

- Sample results should be interpreted as follows

Cutoff	Result	Interpretation
⩾ 30%	Positive	A positive results indicated the presence of SARS-CoV-2 neutralizing antibody.
＜ 30%	Negative	A negative result may indicate absence or level of SARS-CoV-2 neutralizing antibody below the limit of detection of this test. A negative result can also be seen in samples taken during an acute infection prior to antibody seroconversion.

Test results are reported qualitatively as positive or negative. However, diagnosis of SARS-CoV-2 infection should be used in conjugation with clinical findings, patient history, in association with medical judgement and other diagnostic procedures.

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

XV. LIMITATIONS OF THE PROCEDURE

- This test is designed for qualitative detection.
- 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.
- 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.
- Results from antibody testing should not be used as single procedure to diagnose or exclude SARS-CoV-2 infection or to inform infection status

XVI. 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%.

XVII. PERFORMANCE CHARACTERISTICS

Analytical Specificity (Cross-reactivity):

The SARS-CoV-2 Neutralization Antibody detection assay was evaluated for potentially cross-reacting antibodies to other viruses that may cause symptoms similar to SARS-CoV-2 infection, to other organisms that may cause infectious diseases.

No cross-reactivity was observed for the following diseases:

- Influenza A (n=5)
- Influenza A/B IgM (n=2)
- Influenza A IgG (n=2)
- Influenza B IgG (n=2)

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

- HCV (n=5)
- ANA (n=5)
- RSV IgG (n=4)
- RSV IgM (n=3)
- HBsAB (n=5)
- HBc IgM (n=5)
- HIV (n=10)
- hCoV 229E (n=2)
- hCoV OC43 (n=2)
- MERS-CoV (n=2)
- Dengue (n=3)
- Zika (n=1)

However, due to the close relationship of SARS-CoV-1 (n=2) and SARS-CoV-2, there is some level of cross-reactivity between these two viruses.

		Cross Reactant (n=60)
cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit	False Positive	2
	Negative	58
	Specificity	96.7%

* The results show cross-reactivity to anti-SARS-CoV-1 positive samples. No cross-reactivity was observed with any of the hCoV sera tested nor any of the other anti-sera tested in this study.

Sample Type / Matrix Equivalency:

Sample Type / Matrix Equivalency testing was execute, with the combined cohort consisted of samples from normal healthy people (n=40) and samples from RT-PCR confirmed SARS-CoV-2 positive patients (n=30).

Serum and K2 EDTA Plasma results are considered equivalent.

cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit		K2 EDTA Plasma		
		Positive	Negative	% Agreement
Serum	Positive	29	0	100%
	Negative	0	41	100%

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

XVIII. 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

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

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%)

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

XIX. REFERENCES

1. ZHOU Peng, YANG Xinglou. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature, 2020.
2. XUE Xiongyan, ZHU Changlin, HUANG Shaozhen, (2020) Inactivation of 2019 new coronary virus before antibodies detection by different methods. Journal of Southern Medical University.
3. SHI Heshui, HAN Xiaoyu, FAN Yanqing. Radiologic Features of Patients with 2019-n Co V Infection (2020) Journal of Clinical Radiology.
4. NCCLS. 1991. National Committee for Clinical Laboratory Standard. Internal Quality
5. Testing of Reagent Water in the Clinical Laboratory. NCCLS Publication C3-A3.
6. NCCLS. 1997. National Committee for Clinical Laboratory Standard. Preparation and Testing of Reagent Water in the Clinical Laboratory. NCCLS Publication C3-A3.

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

XX. TROUBLESHOOTING

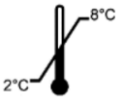
Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Wells are scratched with pipette tip or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates 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 immediately	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

XXI. INSTRUCTION APPROVAL AND REVISION DATE

Approval Date:
Revision Date: 01/02/2021
Date of Issue: 01/08/2021

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District, Nanjing City, Jiangsu Province, 211100, P.R. China

XXII. INDEX OF CE SYMBOLS

REF	Reference Number	IVD	The product is used *in vitro*, please don't swallow it.
	Number of test		Please don't reuse it
	Validity		Please read the instruction book carefully before using
	Warning, please refer to the instruction in the annex	LOT	Batch number
	Date of manufacture		Manufacturer
EC REP	European union authorization representative		Biological risks
	Temperature scope within which the product is reserved	CE	The product meets the basic requirements of European *in vitro* diagnostic medical devices directive 98/79/EC

XXIII. TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

In case of technical problems, you can obtain assistance via contacting the manufacturer below. This product is manufactured by:

Nanjing GenScript Biotech Co., Ltd.
No. 28, Yongxi Road, Jiangning District,
Nanjing City, Jiangsu Province, 211100, P.R. China
Tel: 86-25-58897288-3005
Fax: 86-25-58897288-5815
Website: https://www.genscript.com

EC REP

Lotus NL B.V.
Koningin Julianaplein 10,
1eVerd, 2595AA,
The Hague, Netherlands
Email: peter@lotusnl.com