Version CE.1.0
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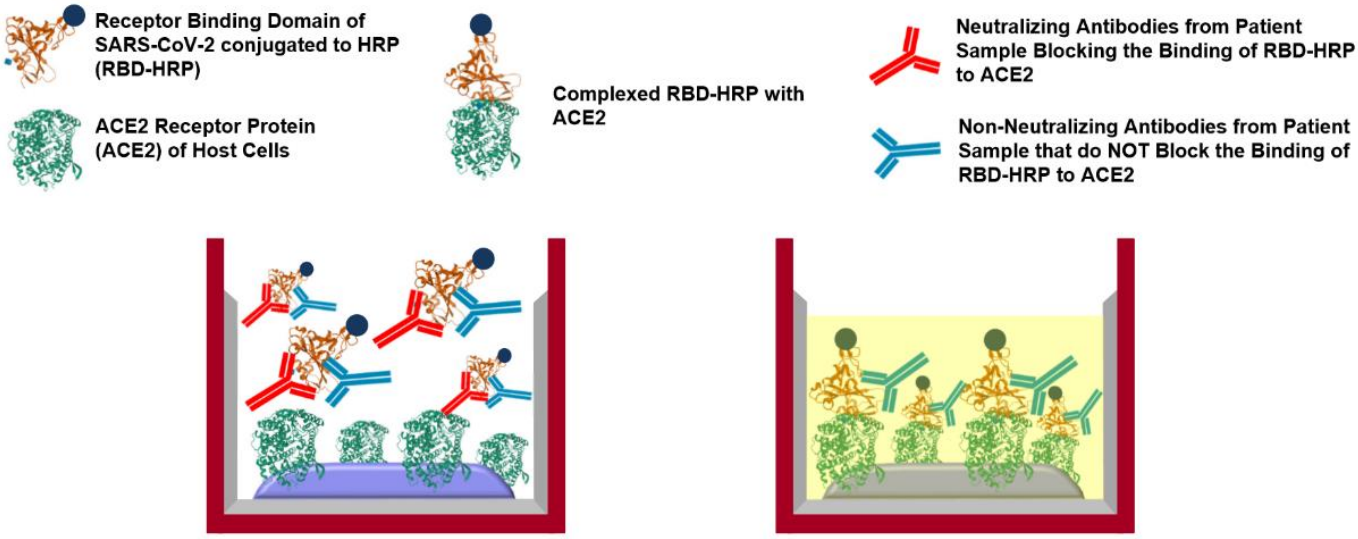
# cPass SARS-CoV-2 Neutralization Antibody Detection Kit

## Instructions for use

REF : L00847 96 Tests

REF : L00847-5 480 Tests




For *In Vitro* Diagnostic Use Only

CE
IVD

Nanjing GenScript Diagnostics Technology Co., Ltd. E-mail: diagnostics@genscript.com

# Contents

## I. INTENDED USE

The cPass SARS-CoV-2 Neutralization Antibody Detection Kit is a Blocking Enzyme-Linked Immunosorbent Assay (ELISA) intended for the qualitative and semi-quantitative direct detection of total neutralizing antibodies to SARS-CoV-2 in human serum and $K_2$-EDTA plasma. The 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 recent or prior infection or vaccination. The cPass SARS-CoV-2 Neutralization Antibody Detection Kit should not be used to diagnose acute SARS-CoV-2 infection.

At this time, it is unknown for how long antibodies persist following infection and if the presence of neutralizing antibodies confers protective immunity. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C 263a to perform high complexity tests.

Results are for the detection of SARS CoV-2 total neutralizing antibodies. Antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time neutralizing antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

The sensitivity of the cPass SARS-CoV-2 Neutralization Antibody Detection Kit early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

## 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 [1]. 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) [1]. 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. Infection with SARS-CoV-2 initiates an immune response, which includes the production of antibodies in the blood. The subset of secreted antibodies that have been demonstrated in a laboratory to prevent SARS-CoV-2 viral entry to human cells are termed neutralizing antibodies [2-7]. Results from standard SARS-CoV-2 serology assays that only detect binding antibodies (such as IgG and total antibody) cannot differentiate between general binding antibodies and neutralizing antibodies.

Neutralizing antibodies to natural SARS-CoV-2 infection are generally detectable in blood several days after initial infection similar to the time frame for production of IgG binding antibodies [17].. Although SARS-CoV-2 infected individuals may have detectable antibodies present for several months following seroconversion [21-23] and the temporal persistence of neutralization antibodies has been shown to decline [24-26], there is no evidence supporting their total duration.

Current serology assays for the measurement of SARS-CoV-2 neutralizing antibodies require the use of live cells, viral components, biosafety level 3 containment facilities and several days to produce results [17]. The cPass SARS-CoV-2 Neutralization Antibody Detection Kit is a neutralizing antibody test for SARS-CoV-2 infection using purified proteins and based on the key viral recognition, docking and infection through the interaction of the SARS-CoV-2 RBD and human ACE2 receptor (hACE2) [27]. The use of purified hACE2 protein coated ELISA plates and HRP-conjugated RBD (RBD-HRP) allows this test kit to assess the presence of circulating antibodies that block the interaction of RBD-HRP with hACE2 with high correlation to the gold standard live cell Plaque Reducing Neutralization Test (PRNT) [17,

27-32].

## III. ASSAY PRINCIPLE

The cPass SARS-CoV-2 Neutralization Antibody Detection Kit is a blocking ELISA test that detects functional immunoglobulins neutralizing the interaction between RBD and hACE2. The kit contains two key components: RBD-HRP and hACE2. The protein-protein interaction between RBD-HRP and hACE2 is disrupted by neutralizing antibodies against SARS-CoV-2 RBD, if present in a clinical sample .

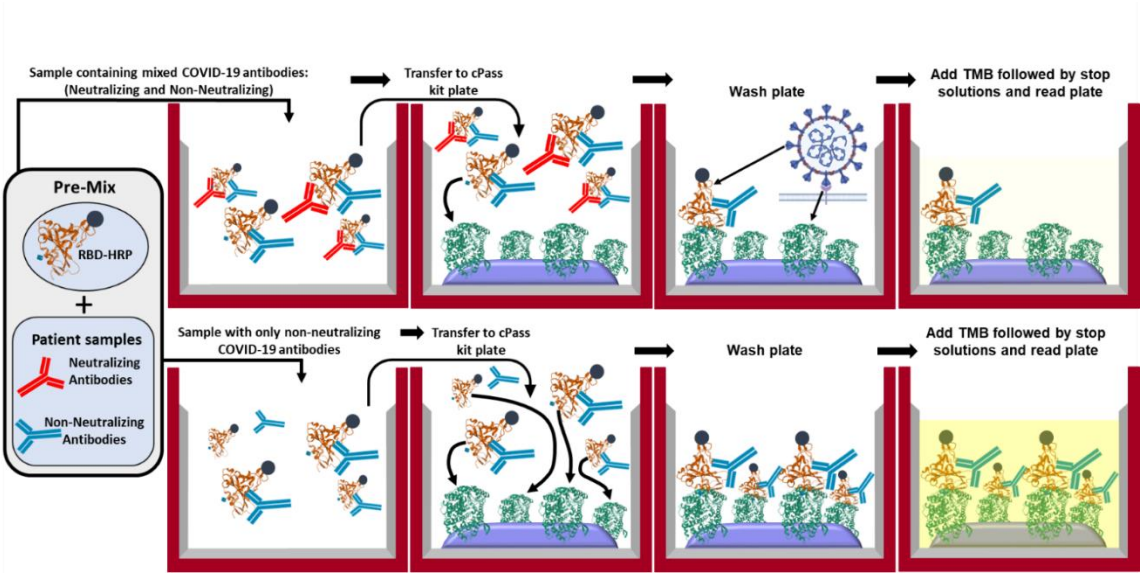



**Figure 1.** Principle of the cPass SARS-CoV-2 Neutralization Antibody Detection Kit. Sample dilutions are initially mixed with the RBD-HRP solution with incubation to permit binding of components to the RBD. If the sample does not contain constituents that bind and block the RBD-hACE2 interaction (bottom four wells) the RBD-HRP will bind to the hACE2-coated wells giving a yellow color after incubation with TMB followed by stop solution. If the sample does contain blocking constituents, they will bind to the RBD and inhibit the interaction with hACE (top four wells) giving a light yellow color after addition of stop solution [17, 27].

First, the samples and controls are pre-incubated with the RBD-HRP to allow the interaction and binding of neutralization antibodies to RBD-HRP (Figure 1). The mixture is then added to the capture plate pre-coated with the hACE2 protein (Figure 1). The unbound RBD-HRP as well as any RBD-HRP bound to non-neutralizing antibody will be captured on the plate. The neutralization antibody complexed to RBD-HRP remains in the supernatant and is removed during washing. After the wash steps, TMB solution is added, giving a blue color. By adding Stop Solution, the reaction is quenched, the color turns yellow and the wells are 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 (Figure 1).

## IV. 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 (0.3 mL) | S1-10 | 1 vial (1.5 mL) | S5-10 |
| Negative Control | 1 vial (0.3 mL) | S1-11 | 1 vial (1.5 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 (15 mL) | S1-90 | 1 bottle (75 mL) | S5-90 |
| Sample Dilution Buffer | 1 bottle (35 mL) | S1-60 | 1 bottle (175 mL) | S5-60 |
| 20× Wash Solution | 1 bottle (40 mL) | S1-70 | 1 bottle (200 mL) | S5-70 |
| TMB Solution | 1 bottle (21 mL) | S1-40 | 1 bottle (105 mL) | S5-40 |
| Stop Solution | 1 bottle (7 mL) | S1-50 | 1 bottle (35 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.

## V. COMPATIBLE AUTOMATION INSTRUMENTS

The cPass SARS-CoV-2 Neutralization Antibody Detection Kit can be used with the automatic machines including the instruments listed below, but not excluding other automated and semiautomated ELISA machines:

- Dynex Agility, DS2
- Aikang Uranus AE 115
- Tecan EVOlyzer
- Hamilton Microlab STAR M

## VI. STORAGE

The unopened kit is stable for one year 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.

## VII. WARNINGS

For *In Vitro* Diagnostic Use only

1. This test has been authorized only for the presence of total neutralizing antibodies against SARS-CoV-2, not for any other viruses or pathogens; and
2. Human source material used to prepare the controls included in this kit should be handled as potentially infectious material. Use universal precautions when handling.
3. Do not pipette by mouth.
4. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
5. Wear disposable gloves while handling the kit reagents and wash hands thoroughly afterwards.
6. Certain components of this product contain 0.03% ProClin 300 as a preservative, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals
7. Certain components are labeled with the following: Irritating to eyes (R 36). Irritating to skin (R 38). Avoid contact with skin (S 24). Avoid contact with eyes (S 25). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S 26). Wear suitable protective clothing (S 36). If swallowed, seek medical advice immediately and show this container or label (S 46).

## VIII. PRECAUTIONS

1. 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.
2. Do not mix components from different batches. Do not mix with components from other manufacturers.
3. Do not use reagents beyond the stated expiration date.

4. All reagents must return to room temperature (20°C to 25°C) before running assay. Use the required volume of reagents only. Do not pour reagents back into vials as reagent contamination may occur.
5. Before opening Positive Control and Negative Control, tap the vial on the benchtop to ensure that all liquid is at the bottom of the vial.
6. Use only distilled or deionized water and clean glassware.
7. Do not let wells dry during test; add reagents immediately after washing steps.
8. The vials Positive Control, Negative Control, HRP conjugated RBD, HPR Dilution Buffer, Sample Dilution Buffer and 20x Wash Solution is labelled

Warning

H317- May cause an allergic skin reaction.

H412 Harmful to aquatic life with long effects.

Contains Mixture of: 5-Chlor-2-methyl-2H-isothiazol.3-on [EG-Nr. 247-500-7] und 2-Methyl-2H-isothiazol-3-on [EG-Nr. 220-239-6] (3:1)

P273 Avoid release to the environment.

P280 Wear protective gloves / protective clothing / eye protection

P302+P352 Dispose of contents/container to ...

P333+P313 IF ON SKIN: Wash with plenty of water.

P501 If skin irritation or rash occurs: Get medical advice/attention.

9. TMB Solution is labelled: EUH210 Safety data sheets available on request.
10. STOP solution is labelled

Warning

H290 - May be corrosive to metals.

Contain: Hydrochloric acid

P234 Keep only in original container/packaging.

P404 Store in a closed container.

P390 Absorb spillage to prevent material damage

## IX. REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

- SARS-CoV-2 Neutralizing Antibody Calibrator (Cat# DXC003) for semi-quantitative analysis of neutralizing antibody titers. 1 vial (0.2 ml), containing neutralizing monoclonal antibody, Phosphate buffer with 2% BSA, 0.1% Proclin-300). Refer to Instructions for Use contained in this product.
- Single or dual wavelength microplate reader with 450nm 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 (or alternatively uncoated 96-well microtiter plates) to aliquot and dilute samples
- 10µL, 200µL and 1000µL precision pipettes
- 10µL, 200µL and 1000µL pipette tips
- Multichannel pipettes
- Disposable reagent reservoir
- Paper towel
- Laboratory timer
- Refrigerator to store samples and kit components
- Centrifuge
- 37ºC Incubator
- 25ºC Incubator

## X. SPECIMEN COLLECTION AND STORAGE

1. Handle all serum and $K_2$-EDTA plasma 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 cPass SARS-CoV-2 Neutralization Antibody Detection Kit, a minimum volume of 30μL per serum or $K_2$-EDTA plasma sample is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture. Early separation from the clot prevents hemolysis of serum.
4. For human 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 plasma, treat blood with the anticoagulant $K_2$-EDTA. 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.

## XI. SEMI-QUANTITATIVE PROTOCOL

### Reagent Preparation

1. All reagents must be taken out from refrigeration and allowed to equilibrate at room temperature (20° to 25°C) before use. Save all reagents in refrigerator promptly after use.
2. All samples and controls should be vortexed before use. Briefly centrifuge to assure all reagents are at the bottom of the tubes for accurate pipetting.
3. **RBD-HRP Solution**: Dilute HRP conjugated RBD 1:1000 with RBD Dilution Buffer. For example, dilute 10µL of HRP conjugated RBD with 10mL of HRP Dilution Buffer to produce the 1X RBD-HRP solution.
4. **Stock SARS-CoV-2 Neutralizing Antibody Calibrator (GenScript #DXC003)**: The SARS-CoV-2 Neutralizing Antibody (NAb) Calibrator is supplied in a **Stock** solution at a concentration of $1\times10^6$ Units (U) per mL (U/mL) (Figure 2). Produce a **Diluted Stock** solution of 6000U/mL by mixing 6µL of the **Stock** with 994µL of the kit supplied Sample Dilution Buffer (Figure 2). Each 30µL of the **Diluted Stock** is enough to run the NAb dilution series in duplicate on each plate (Figure 2). Store the **Diluted Stock** of NAb in aliquots frozen at -20ºC.
5. **1× Wash Solution**: Dilute the 20X Wash Solution from kit L00847 with deionized or distilled water with a volume ratio of 1:20. For example, dilute 40mLs of 20X Wash Solution with 760mLs of deionized or distilled water to make 800mLs of 1X Wash Solution. Store the solution at 2°C to 8°C.

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

### Sample, Positive/Negative Control and SARS-CoV-2 Neutralizing Antibody Callibrator Preparation

- **Sample Dilutions:** Dilute the test samples 1:10 in Sample Dilution Buffer, taking in consideration samples should be tested in duplicates.

- **Positive/Negative Control Dilutions:** Dilute the positive and negative controls 1:10 by mixing 7µL of control with 63µL of Sample Dilution Buffer. The controls can be diluted into a 96-well microtiter plate containing the diluted samples, controls and standards to streamline the pipetting and minimize the time for the downstream steps.
- **SARS-CoV-2 Neutralizing Antibody Calibrator Working Solution (monoclonal antbodies with neutralization activity to SARS-CoV-2):** Dilute the 6000 U/mL **Diluted Stock** (see Step 4 in Reagent Preparation section above) by a factor of 1:10 to a 600U/mL **Working Solution** by adding 30µL of the **Diluted Stock** solution to 270µL of Sample Dilution Buffer (Figure 2).

**SARS-CoV-2 Neutralizing Antibody Calibration Curve Preparation (see Figure 2)**

The calibrators can be diluted into a 96-well microtiter plate containing the diluted samples, controls and calibrators to streamline the pipetting and minimize the time for the downstream steps. The calibration curve from the **neutralizing antibody calibrator working solution** (described above) is prepared according to the steps below as depicted in Figure 2:

1. The 300uL, **SARS-CoV-2 Neutralizing Antibody Calibrator Working Solution** (see above) represents a final concentration of 600U/mL. Label this tube “1A”. Vortex and lightly centrifuge to assure proper mixing.
2. Serially dilute the 600U/mL **Working Solution** (Tube 1A) by a factor of 1:2 for six dilutions in Sample Dilution Buffer according to Figure 2 as follows:
    a. Prepare six, 1.5ml Eppendorf tubes labelled alphanumerically from “1B” to “1G” consecutively and one additional tube labelled 1H for Background.
    b. Pipette 150µL of **Sample Dilution Buffer (Diluent)** into each of tubes 1B through 1H using a calibrated P200 pipette.
    c. Transfer 150µL from Tube 1A to Tube 1B using a calibrated P200 pipette then vortex and lightly centrifuge. Transfer 150µL from Tube 1B to Tube 1C and vortex/centrifuge. Continue the serial 1:2 dilution series by transferring 150µL from Tube 1C to Tube 1D with vortex/centrifugation. Complete the serial dilutions from Tube 1D to Tube 1E, Tube 1E to Tube 1F and Tube 1F to Tube 1G always

with vortexing and centrifugation between each transfer to assure adequate and uniform mixing.

Up and down pipetting at least three times can substitute for vortexing to mix standard dilutions.

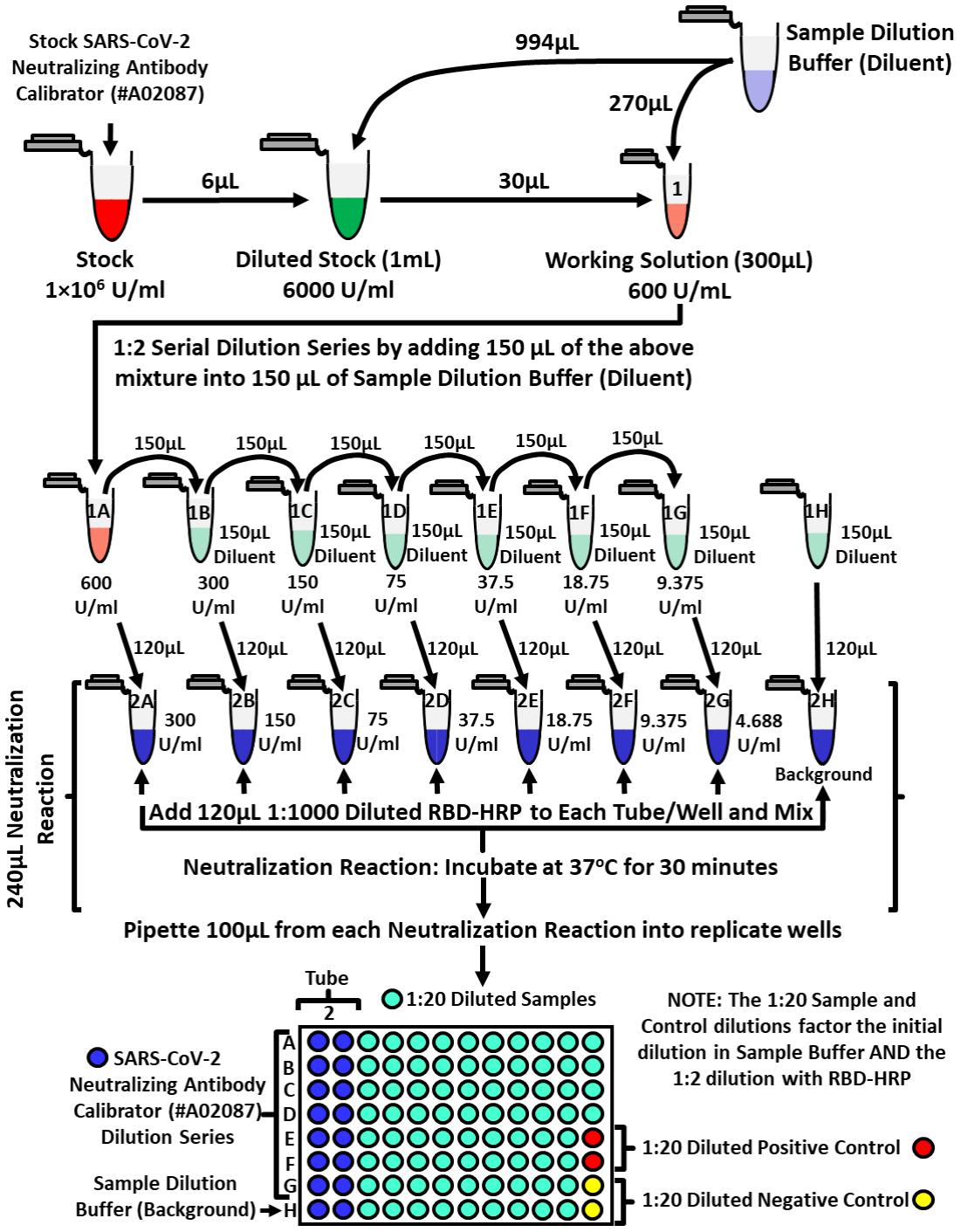



**Figure 2.** SARS-CoV-2 Neutralizing Antibody (NAb) calibration (GenScript #DXC003) curve and plating schematic. The antibody and all dilutions should be stored at -20°C.

**Samples, SARS-CoV-2 Neutralizing Antibody Calibrator, Controls and ACE2-coated Capture Plate Preparation**

1. It is recommended that all positive/negative controls, SARS-CoV-2 neutralizing antibody calibrator and samples should be prepared in duplicate in a 96-well microtiter plate to streamline the pipetting and minimize the time to prepare the neutralization reactions.
2. Make sure the strips for the ACE2-coated assay plate are tightly snapped into the plate frame.
    a) 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 damaging the Capture Plate.

■ **Test Procedure (Warning: The procedure contains guidance for running the cPass assay with automated liquid handling and ELISA systems. The selection of the incubation temperature will depend on your specific instrument capabilities. Please consult with GenScript technical support for appropriate validation procedures to assure robust and reproducible data using automation with the cPass assay.)**

**Neutralization Reaction Mixtures (SARS-CoV-2 Neutralizing Antibody Calibrator Curve, Samples and Controls)**

1. Prepare eight, 1.5ml Eppendorf tubes labelled alphanumerically from “2A” to “2H” consecutively. Transfer 120µL from each of the SARS-CoV-2 Neutralizing Antibody Calibrator Curve dilution tubes (“1A” to “1H”) to the corresponding tubes “2A” to “2H” according to Figure 2 above. Alternatively, 120µL of the diluted standards can be added directly into the first column of a 96-well microtiter “Neutralization Reaction” plate containing 60µL of the 1:10 diluted samples and controls to streamline the pipetting and minimize the time for the downstream steps.
2. Add 120µL of the 1:1000 diluted RBD-HRP Solution (see “**Reagent Preparation**” subsection above) to each of tubes “2A” through “2H” (or columns A1 to H1 in a

microtiter plate) and mix with up and down pipetting two times. This will result in 240µL of **Neutralization Reaction** solution for each SARS-CoV-2 neutralizing antibody calibrator curve dilution (2A through 2G) and the associated "Background" (2H) (or columns A1 to H1 in a microtiter plate). Also, combine 60µL of the 1:10 diluted samples, positive and negative controls with 60µL of the 1:1000 diluted HRP-RBD solution (see "**Reagent Preparation"** subsection above) and mix with up and down pipetting two times. The neutralization reactions for the diluted samples, controls and standards can be prepared in a 96-well, microtiter plate to streamline the pipetting and minimize the time for the downstream steps.

3. For manual sample processing, incubate the mixtures at 37°C for 30 minutes. For automated sample processing, incubate the mixtures at 37°C for 30 minutes or at room temperature for 45 minutes. For both manual and automated processing, start timing after the addition of HRP-RBD solution to the first well such that all wells are incubating for the same total time.

**Interaction of Free RBD-HRP with ACE2**

1. Add 100µL of the RBD-HRP **Neutralization Reaction Mixtures** above to the corresponding wells of the ACE2-coated assay microtiter plate (Figure 2).
2. For manual sample processing, incubate at 37°C for 15 minutes. For automated sample processing, incubate the mixtures at 37°C for 15 minutes or at room temperature for 20 to 25 minutes. For both manual and automated processing, start timing after the addition of RBD-HRP neutralization mixtures to the first well.
3. Wash the plate four times with 260µL of 1× Wash Solution assuring that the first wash cycle accounts for the lag time in pipetting the neutralization reactions into the ACE2-coated plate.
4. Pat the plate on paper towel to remove residual liquid in the wells after washing steps.

**Substrate Reaction and Absorbance Measurement**

1. For manual sample processing, add 100µL of TMB Solution to each well and incubate

the plate in dark at 25°C for 15 minutes. For automated sample processing, add between 100µL and 200 µL of TMB Solution to each well and incubate the plate at 25°C for 15 minutes or at room temperature for 18 to 25 minutes. For both manual and automated processing, start timing after the addition of TMB Solution to the first well..

2. To assure all reactions are incubated with the TMB solution for the same time, add 50µL of Stop Solution to each well in the same consecutive order with the same pipette and pipetting technique as the TMB solution to quench the reactions.
3. Read the absorbance in a microtiter plate reader at 450nm immediately.

## XII. CRITICAL PIPETTING CONSIDERATIONS TO ASSURE QUALITY RESULTS

To achieve high quality results, the pipetting and incubation times per sample should be precise. To this end, the following recommendations should be followed:

1. Pre-dilute the samples, controls and the SARS-CoV-2 Neutralizing Antibody Calibration Curve (Figure 2) into a 96-well microtiter plate (Sample Plate).
2. Cover the plate and mix using a standard plate shaker OR by up and down pipetting in the sample plate at least three times. If required perform a quick spin of the plate in a centrifuge to assure all the liquid is at the bottom of the wells.
3. Prepare the 1:1000 RBD-HRP and add to a multi-channel, pipette trough for easy transfer of the solution by multi-channel or repeater pipetting.
4. Use an eight or twelve channel, multi-channel pipette to transfer 60uL of the pre-diluted samples and controls from the Sample Plate into a second 96-well, microtiter plate (Neutralizing Reaction Plate). Then transfer 60µL of the 1:1000 diluted RBD-HRP solution from the pipette trough into each of the sample and control wells of the Neutralizing Reaction Plate to generate 120µL neutralization reaction mixtures (Figure 2). Also, prepare the 240µL of **SARS-CoV-2 Neutralizing Antibody Calibration Curve** neutralization reactions (Figure 2) in the first column of the

neutralization reaction plate. With a multi-channel pipette, it should take less than two minutes to produce the neutralization reaction plate with the calibrators, samples and controls.

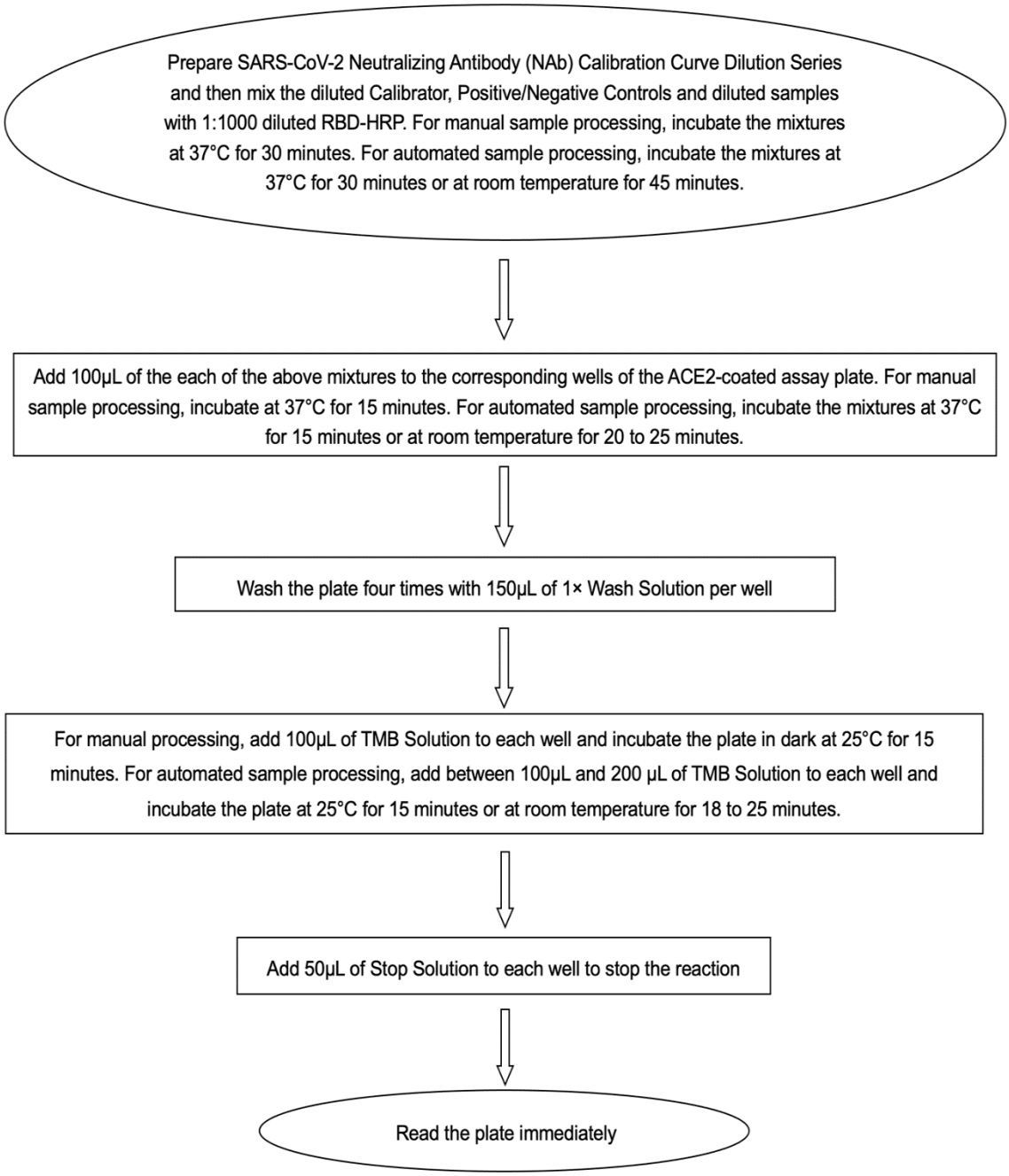
5. For manual sample processing, incubate the mixtures at 37°C for 30 minutes. For automated sample processing, incubate the mixtures at 37°C for 30 minutes or at room temperature for 45 minutes. For both manual and automated processing, start timing after the addition of HRP-RBD solution to the first well such that all wells are incubating for the same total time. The preparation of the Neutralization Reaction Plate should not require more than about 2 minutes upon which the plate should be immediately placed in the temperature-controlled incubator. Presuming this is the case, start timing upon incubation.
6. With a multichannel pipette, transfer 100µL of the neutralizing reaction mixture from each well of the Neutralizing Reaction Plate into the opposing wells of the ACE2-coated Assay Plate assuring that the first two columns are reserved for the standard curve if semi-quantitative analysis is applied. This transfer should take less than two minutes with a multi-channel pipette.
7. Incubate the ACE2-coated assay plate at 37°C for 15 minutes (manual/automated protocols) or at room temperature for 20 to 25 minutes (automated protocol). For both manual and automated processing, start timing after the addition of RBD-HRP neutralization mixtures to the first well.
8. Wash the ACE2-coated Assay Plate four times with 260µL of 1× Wash Solution by inverting the plate and dumping the Neutralization Reaction mixtures. Then use a multichannel or repeater pipette to add the wash solution to each well. This process should take less than 2 minutes.
9. Add the TMB to a pipette trough and transfer the solution by multichannel or repeater pipetting into the ACE2 coated assay plate. This process should take less than 2 minutes.
10. For manual sample processing, add 100µL of TMB Solution to each well and incubate the plate in dark at 25°C for 15 minutes. For automated sample processing, add

between 100µL and 200 µL of TMB Solution to each well and incubate the plate at 25°C for 15 minutes or at room temperature for 18 to 25 minutes. For both manual and automated processing, start timing after the addition of TMB Solution to the first well. The addition of TMB should not require more than about 2 minutes upon which the plate should be immediately placed in the temperature-controlled incubator. Presuming this is the case, start timing upon incubation at 25°C.

11. Add the stop solution to a pipette trough and transfer the solution by multichannel or repeater pipetting to the assay plate. This process should not take more than two minutes.
12. Then immediately read the plate at 450nm.

All multi-channel pipetting and wash steps should be performed in the same order of wells and in the same approximate time frame to assure uniformity in well-to-well incubation times for each step in the protocol.

## ASSAY PROCEDURE SUMMARY



## 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 the controls must fall within the values 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.

**Pre-established OD450 values for quality control**

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

## XIV. INTERPRETATION OF RESULTS

The cutoff value for the cPass SARS-CoV-2 Neutralizing Antibody Detection Kit is 30% signal inhibition. The percent signal inhibition for the detection of neutralizing antibodies are calculated from the formula below.

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



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Please follow the table below to determine the results to be reported outside the laboratory:

| cPass SARS-CoV-2 Neutralization Antibody Detection Kit | | | | |
|---|---|---|---|---|
| **Qualitative** | | **Semi-Quantitative** | **Final Results Reported**** | |
| **% Signal Inhibition** | **Result** | **Result (Units/mL Value)** | **Result** | **Interpretation*** |
| **X < 30%** | Negative | N/A | Negative | Neutralizing antibodies to SARS-CoV-2 are NOT detected |
| **X ≥ 30%** | Positive | X < 47 | Positive | Neutralizing antibodies to SARS-CoV-2 are detected. The measured value is reported as "<47 Units/mL". |
| | | <100 U/mL | Low Titer | Low level of neutralizing antibodies to SARS-CoV-2 are detected. |
| | | ⩾100U/mL | High Titer | High level of neutralizing antibodies to SARS-CoV-2 are detected. |

*The cPass Neutralization Antibody Detection Kit results have shown 95.7% PPA (95.% CI [85.8 - 98.8] %) and 97.8% NPA (95% CI 92.5 – 99.4]%) with 50% viral neutralization by PRNT in clinical study.

## XV. LIMITATIONS OF THE PROCEDURE

- This test is designed for both qualitative and semi-quantitative detection of SARS-CoV-2 neutralizing antibodies.
- Use of cPass SARS-CoV-2 Neutralization Antibody detection Kit is limited to laboratory personnel who have been trained. Not for home use.
- Performance has only been established with the specimen types listed in the Intended Use. Other specimen types have not been evaluated and should not be used with this assay.
- Negative results do not rule out SARS-COV-2 infection, particularly those who have been in contact with the virus. Direct testing with a molecular diagnostic should be performed to evaluate for acute SARS-CoV-2 infection in symptomatic individuals.
- Positive results may be due to current or past infection with non-SARS-COV-2 corona virus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
- Results from this test should not be used to diagnose or to exclude acute SARS-COV-2 infection or to inform infection status.
- A positive result may not indicate previous SARS-CoV-2 infection. Consider other information including clinical history and local disease prevalence, in assessing the need for a second but different serology test to confirm an immune response.
- The cPass SARS-CoV-2 Neutralization Antibody Detection Kit is known to cross-react with SARS-CoV-1 neutralizing antibodies.

## XVI. POTENTIAL CROSS-REACTIVITY

To evaluate the potential cross-reactivity of the cPass SARS-CoV-2 Neutralization Antibody Detection Kit, positive and negative controls and 60 clinical specimen seropositive for other diseases were tested in duplicate. The table below summarizes the results with the cPass SARS-CoV-2 Neutralization Antibody Detection Kit table for wet tested organisms:

| Samples | Seropositive for Disease | cPass SARS-CoV-2 Neutralization Antibody Detection Kit | | |
|---|---|---|---|---|
| | | %CV | Mean Value Result (% signal inhibition) | Result Reported (Positive ≥ 30%) |
| 1 | Influenza A | 1% | 8% | Negative |
| 2 | Influenza A | 3% | 5% | Negative |
| 3 | Influenza A | 0% | -9% | Negative |
| 4 | Influenza A | 2% | 1% | Negative |
| 5 | Influenza A | 3% | 5% | Negative |
| 6 | Influenza A/B IgM | 3% | -1% | Negative |
| 7 | Influenza A IgG | 4% | -7% | Negative |
| 8 | Influenza B IgG | 4% | 7% | Negative |
| 9 | Influenza A IgG | 7% | 1% | Negative |
| 10 | Influenza A/B IgM | 4% | -8% | Negative |
| 11 | Influenza B IgG | 2% | 2% | Negative |
| 12 | HCV | 1% | 4% | Negative |
| 13 | HCV | 1% | 3% | Negative |
| 14 | HCV | 5% | -1% | Negative |
| 15 | HCV | 4% | 0% | Negative |
| 16 | HCV | 1% | 4% | Negative |
| 17 | ANA | 1% | 12% | Negative |
| 18 | ANA | 4% | -2% | Negative |
| 19 | ANA | 3% | -11% | Negative |
| 20 | ANA | 1% | -1% | Negative |
| 21 | ANA | 3% | 3% | Negative |
| 22 | RSV IgG | 2% | -7% | Negative |
| 23 | RSV IgG | 4% | -10% | Negative |
| 24 | RSV IgG | 1% | -4% | Negative |
| 25 | HBsAB | 3% | -4% | Negative |
| 26 | HBsAB | 0% | -8% | Negative |
| 27 | HBsAB | 1% | -9% | Negative |
| 28 | HBsAB | 2% | -3% | Negative |
| 29 | HBc IgM | 4% | -1% | Negative |
| 30 | HBc IgM | 0% | -6% | Negative |
| 31 | HBc IgM | 3% | -4% | Negative |

| | | | | |
|---|---|---|---|---|
| 32 | HBc IgM | 1% | -1% | Negative |
| 33 | HBc IgM | 8% | -4% | Negative |
| 34 | HBsAB | 3% | -11% | Negative |
| 35 | RSV IgG | 0% | -2% | Negative |
| 36 | RSV IgM | 2% | -5% | Negative |
| 37 | RSV IgM | 1% | -5% | Negative |
| 38 | RSV IgM | 3% | -10% | Negative |
| 39 | HIV | 7% | 16% | Negative |
| 40 | HIV | 10% | 7% | Negative |
| 41 | HIV | 4% | 6% | Negative |
| 42 | HIV | 7% | 7% | Negative |
| 43 | HIV | 0% | 19% | Negative |
| 44 | HIV | 3% | 13% | Negative |
| 45 | HIV | 6% | 10% | Negative |
| 46 | HIV | 4% | 13% | Negative |
| 47 | HIV | 2% | 17% | Negative |
| 48 | HIV | 9% | 12% | Negative |
| 49 | hCoV 229E | 0% | 10% | Negative |
| 50 | hCoV 229E | 0% | 10% | Negative |
| 51 | hCoV OC43 | 2% | 11% | Negative |
| 52 | hCoV OC43 | 12% | 9% | Negative |
| 53 | SARS-CoV-1* | 4% | 36% | Positive |
| 54 | SARS-CoV-1* | 8% | 59% | Positive |
| 55 | MERS-CoV | 5% | 12% | Negative |
| 56 | MERS-CoV | 3% | 12% | Negative |
| 57 | Dengue | 4% | -1% | Negative |
| 58 | Dengue | 3% | 2% | Negative |
| 59 | Dengue | 4% | 1% | Negative |
| 60 | Zika | 1% | 7% | Negative |

* 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.

## XVII. CLINICAL PERFORMANCE

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

**Table 1: $PRNT_{50}$:**

| 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. |

**Table 2: $PRNT_{90}$:**

| 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. |

**STUDY 1:**

The first 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 cPass SARS-CoV-2 Neutralization Antibody Detection Kit sample results were compared to a Plaque Reduction Neutralization Test performed to WHO guidelines. Tables 3 and 4 show the Positive and Negative Percent Agreement between the $PRNT_{50}$ or $PRNT_{90}$ and the cPass SARS-CoV-2 Neutralization Antibody Detection Kit results when evaluating samples collected from RT-PCR positive and negative individuals.

**Table 3: 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%) |

**Table 4: 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%) |

**STUDY 2:**

The second clinical agreement study evaluated a total of 140 samples retrospectively collected from SARS-COV-2 RT-PCR positive individuals using the cPass SARS-CoV-2 Neutralization Antibody Detection Kit. The cohort consisted of 93 $PRNT_{50}$ negative samples and 40 $PRNT_{50}$ positive samples. The cPass SARS-CoV-2 Neutralization Antibody Detection Kit results were compared to a $PRNT_{50}$ comparator test. Overall PPA and NPA are shown in Table 5 below:

**Table 5: Clinical Agreement using $PRNT_{50}$ titers as the comparator method**

| | | Plaque Reduction Neutralization Test ($PRNT_{50}$) | |
|---|---|---|---|
| | | Positive (n=47) | Negative (n=93) |
| GenScript cPass SARS-CoV-2 Neutralization Antibody Detection Kit | Positive | 45 | 2 |
| | Negative | 2 | 91 |
| | Positive Percent Agreement and 95% CI | 45/47= 95.7% (95% CI 85.8 – 98.8 %) | |
| | Negative Percent Agreement And 95% CI | | 91/93= 97.8% (95% CI 92.5 – 99.4 %) |

Additional semi-quantitative data analysis show concordance between the cPass SARS-CoV-2 Neutralization Antibody Detection kit results and the titers obtained with the neutralization comparator method (PRNT50) as shown in Table 6 below.

**Table 6: Concordance between cPass Neutralization Antibody Detection kit and PRNT50 titers**

| | | | **$PRNT_{50}$** | | |
|---|---|---|---|---|---|
| | **Analyte Level Categories** | | | **Low Titer** | **High Titer** |
| | | Titers Sub-Categories | Target Not Detected < 1:20 | 1:40-1:320 | 1:640-5120 |
| **cPass SARS-CoV-2 Neutralization Antibody Detection Kit interval of numerical values** | **Negative** | Target Not Detected <30% Inhibition | 91 | 2 | 0 |
| | **Low Titer** | <100U/mL | 2 | 22 | 1 |
| | **High Titer** | ≥100U/mL | 0 | 5 | 17 |
| **Total** | | | **93** | **29** | **18** |
| **Exact Agreement per Neutralization Titer Category** | **Equation** | | | **22/29=75%** | **17/18=94.4%** |
| | **Acceptance Criteria** | | | 60% | 60% |
| **+/ -Agreement per Neutralization Titer Category** | **Equation** | | | **27/29=93%** | **18/18=100%** |
| | **Acceptance Criteria** | | | 80% | 80% |
| | **95% Score CI** | | | 78.0-98.1% | 82.4-100% |
| **Negative Percent Agreement (NPA)** | **Equation** | | **91/93= 98%** | | |
| | **Acceptance Criteria** | | 95% | | |
| | **95% Score CI** | | 92.5-99.4% | | |

- 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.
- 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.

## XVIII. PRECISION

To evaluate the Precision of the cPass SARS-CoV-2 Neutralization Antibody Detection Kit a weak, moderate and high positive samples were used together with the test Positive control. Two sites were included in this study. Per site, the precision testing was performed over 3 days with 2 runs per day and 4 replicate measurement per run for each sample, and 2 replicates per run for the control. In addition, 2 reagent lots and 2 calibrator lots were used (one lot of reagent and 1 lot of calibrator per site). Tables 7 and 8 represent the within-laboratory Precision results per site. Table 9 summarizes the Reproducibility results (between laboratory precision)

**Table 7: Within-Laboratory Precision study (Site 1)**

| Level | N | Mean (Units/mL) | Repeatability | | Between Run | | Between Day | | Within Lab Precision | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 – Weak Positive | 24 | 33.846 | 2.688 | 7.9% | 5.185 | 15.3% | 0.000 | 0.0% | 5.840 | 17.3% |
| 2 – Moderate Positive | 24 | 69.850 | 1.956 | 2.8% | 7.209 | 10.3% | 0.000 | 0.0% | 7.469 | 10.7% |
| 3 – Strong Positive | 24 | 145.625 | 3.692 | 2.5% | 11.574 | 7.9% | 0.000 | 0.0% | 12.149 | 8.3% |
| 4 – Positive Control | 12 | 377.000 | 9.078 | 2.4% | 73.174 | 19.4% | 0.000 | 0.0% | 73.735 | 19.6% |

**Table 8: Within-Laboratory Precision study (Site 2)**

| Level | N | | Repeatability | | Between Run | | Between Day | | Within Lab Precision | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean (Units/mL) | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 – Weak Positive | 24 | 39.296 | 4.966 | 12.6% | 3.024 | 7.7% | 3.232 | 8.2% | 6.652 | 16.9% |
| 2 – Moderate Positive | 24 | 85.650 | 2.305 | 2.7% | 14.257 | 16.6% | 0.000 | 0.0% | 14.442 | 16.9% |
| 3 – | 24 | 187.396 | 4.268 | 2.3% | 6.941 | 3.7% | 8.000 | 4.3% | 11.419 | 6.1% |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Strong Positive | | | | | | | | | | |
| 4 – Positive Control | 24 | 416.142 | 2.043 | 0.5% | 7.344 | 1.8% | 42.586 | 10.2% | 43.263 | 10.4% |

**Table 9: Reproducibility (between Laboratory precision)**

| Level | N | Mean (Units/mL) | Repeatability | | Between Run | | Between Day | | Between Site | | Reproducibility | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 | 47 | 36.957 | 5.070 | 13.72% | 6.197 | 16.77% | 3.564 | 9.64% | 3.859 | 10.44% | 7.300 | 19.75% |
| 2 | 48 | 77.75 | 9.105 | 11.71% | 11.425 | 14.69% | 6.902 | 8.88% | 10.271 | 13.21% | 15.363 | 19.76% |
| 3 | 48 | 166.51 | 8.907 | 5.35% | 15.208 | 9.13% | 12.326 | 7.40% | 28.609 | 17.18% | 32.399 | 19.46% |
| 4 | 24 | 396.558 | 42.965 | 10.83% | 63.138 | 15.92% | 46.266 | 11.67% | 0.000 | 0.00% | 63.138 | 15.92% |

## XIX. 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/Low 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 |

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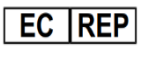
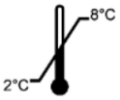
## XXI. INSTRUCTION APPROVAL AND REVISION DATE

Approval Date: 2022.02.17

Revision Date: 2022.02.17

Date of Issue: 2022.02.17

## XXII. INDEX OF CE SYMBOLS

| | | | |
|---|---|---|---|
|  | The product is used in vitro, please don't swallow it. |  | Please don't reuse it |
|  | Validity |  | Please read the instruction book carefully before using |
|  | Warning, please refer to the instruction in the annex |  | Batch number |
|  | Date of manufacture |  | Manufacturer |
|  | European union authorization representative |  | Biological risks |
|  | Temperature scope within which the product is reserved |  | The product meets the basic requirements of European in vitro diagnostic medical devices directive |

| | | | |
|---|---|---|---|
| | | | 98/79/EC |
|  | Warning sign |  | Corrosive to metals |

## 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 Diagnostics Technology Co., Ltd.
Address: 2nd Floor, Unit D, Building 5, Ruihong Zhihui Park, No. 2289 Tianyuan East Road (Jiangning High-tech Park),.Jiangning District, Nanjing City, Jiangsu Province, China
E-mail: diagnostics@genscript.com

EC REP

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