Version 7.0
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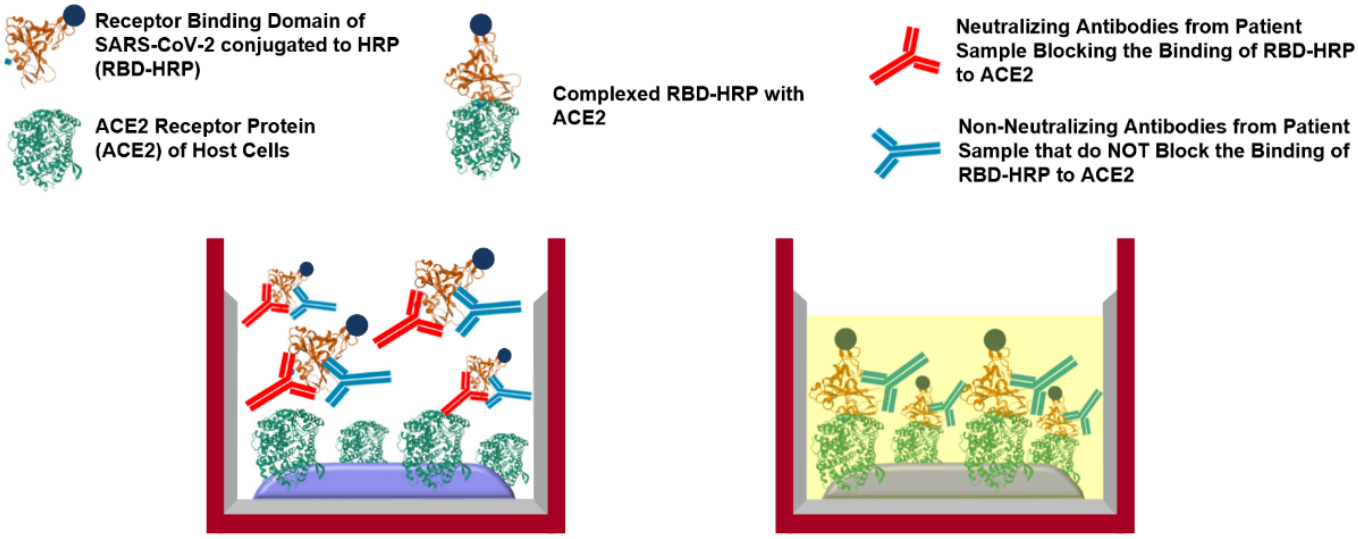
cPass SARS-CoV-2 Neutralization Antibody Detection Kit

Instructions for use

REF : L00847 96 Tests

REF : L00847-5 480 Tests



For *In Vitro* Diagnostic Use Only

For FDA Emergency Use Authorization Only

For Prescription Use Only

The results of this semi-quantitative test should not be interpreted as an indication or degree of immunity or protection from infection.

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

For *In Vitro Diagnostic Use* under Emergency Use Authorization

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 dipotassium 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. The cPass SARS-CoV-2 Neutralization Antibody Detection Kit should not be used to diagnose or exclude 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 that meet requirements 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.

The cPass SARS-CoV-2 Neutralization Antibody Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization

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, live virus, biosafety level 3 containment facilities (if life SARS-CoV-2 virus is used) 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 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 .

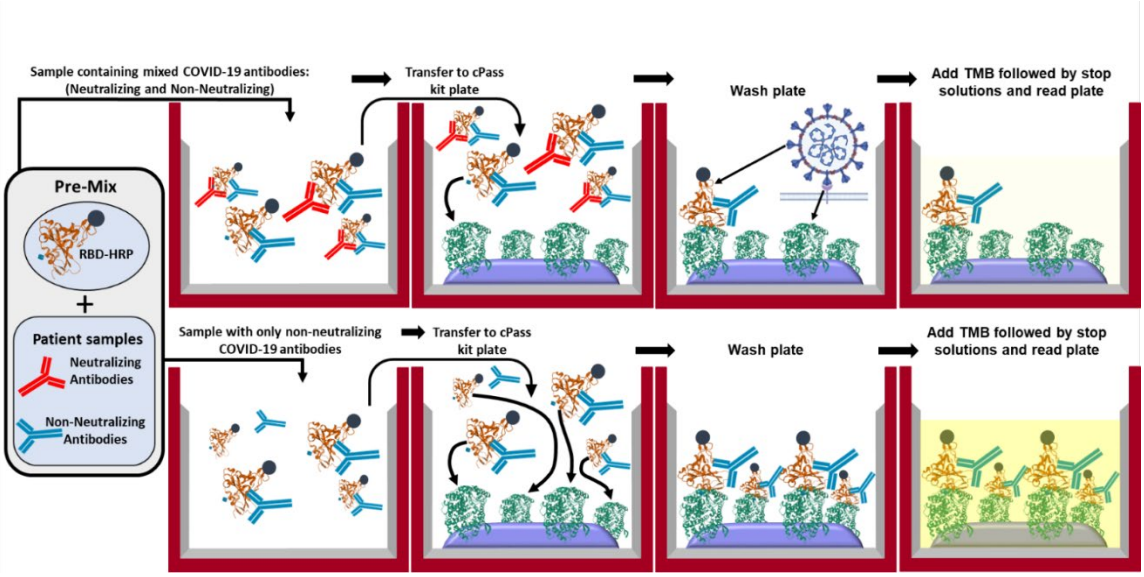


Figure 1. Principle of the cPass SARS-CoV-2 Neutralization Antibody Detection Kit. Sample dilutions are initially mixed with the RBD-HRP solution with incubation for 30 minutes at 37°C to permit binding of components to the RBD. If the sample does not contain SARS-CoV-2 neutralizing antibodies that bind and block the RBD-hACE2 interaction (bottom four wells) the RBD-HRP will bind to the hACE2-coated wells during a 15 minute incubation at 37°C giving a yellow color after incubation with TMB for 15 minutes at 25oC followed by stop solution. If the sample does contain SARS-CoV-2 neutralizing antibodies, they will bind to the RBD during the initial 30 minutes 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). The test is calibrated for the semi-quantitative detection of anti-SARS-CoV-2 neutralizing antibodies using the SARS-CoV-2 Neutralizing Antibody Calibrator (available separately). Semi-quantitative results are expressed in Units/mL.

IV. KIT CONTENTS

Component	96 Tests		480 Tests	
	Quantity	REF	Quantity	REF
Capture Plate*	1 plate	S1-80	5 plates	S5-80
Positive Control (containing SARS-CoV-2 neutralizing antibodies)	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 (RBD-HRP)	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	1 bottle (150 mL)	S5-60
20X Wash Solution	1 bottle (40 mL)	S1-70	1 bottle (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.

V. STORAGE

The unopened kit is stable at 2°C to 8°C until expiration date and the opened kit is stable for up to 1 month from the date of opening at 2°C to 8°C.

VI. WARNINGS

For Prescription and *In Vitro* Diagnostic Use only

1. For Use under an Emergency Use Authorization Only;
2. This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories;
3. This product has been authorized only for detecting the presence of total neutralizing antibodies to SARS-CoV-2, not for any other viruses or pathogens;
4. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
5. Human source material used to prepare the controls included in this kit should be handled as potentially infectious material. Use universal precautions when handling.
6. Do not pipette by mouth.
7. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
8. Wear disposable gloves while handling the kit reagents and wash hands thoroughly afterwards.
9. 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

10. 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).

VII. 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. 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. 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 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.
6. 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.
7. Use only distilled or deionized water and clean glassware.
8. Do not let wells dry during test; add reagents immediately after washing steps.

VIII. REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

- SARS-CoV-2 Neutralizing Antibody Calibrator (Cat# A02087). 1 vial (20 µl), 1,000,000 U/ml, containing SARS-CoV-2 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

IX. 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.

X. 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. Store 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 HRP 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 #A02087)**: The SARS-CoV-2 Neutralizing Antibody (NAb) Calibrator is supplied in a **Stock** solution at a concentration of 1×10^6 Units (U) per mL (U/mL) (Figure 2). Prepare 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 -20°C.
5. **1× Wash Solution**: Dilute the 20X Wash Solution (Ref 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 Calibrator Preparation

- **Sample Dilutions:** Dilute the test samples 1:10 in Sample Dilution Buffer, taking in consideration samples should be tested in duplicates. For example, dilute 7µL of sample with 63µL of Sample Dilution Buffer for singlicate wells. The diluted samples can be placed directly into a 96-well PCR plate containing the diluted samples, controls and standards to streamline the pipetting and minimize the time for the downstream steps. Samples should be tested in duplicate.
- **Positive/Negative Control Dilutions:** Dilute the positive and negative controls 1:10 by mixing 7µL of control with 63µL of Sample Dilution Buffer. The diluted controls can be placed directly into a 96-well PCR plate containing the diluted samples, controls and calibrators to streamline the pipetting and minimize the time for the downstream steps. Controls should be tested in duplicate.
- **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). Calibrator dilutions should be tested in duplicate.

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

The calibrator dilutions can be placed directly into a 96-well PCR 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.

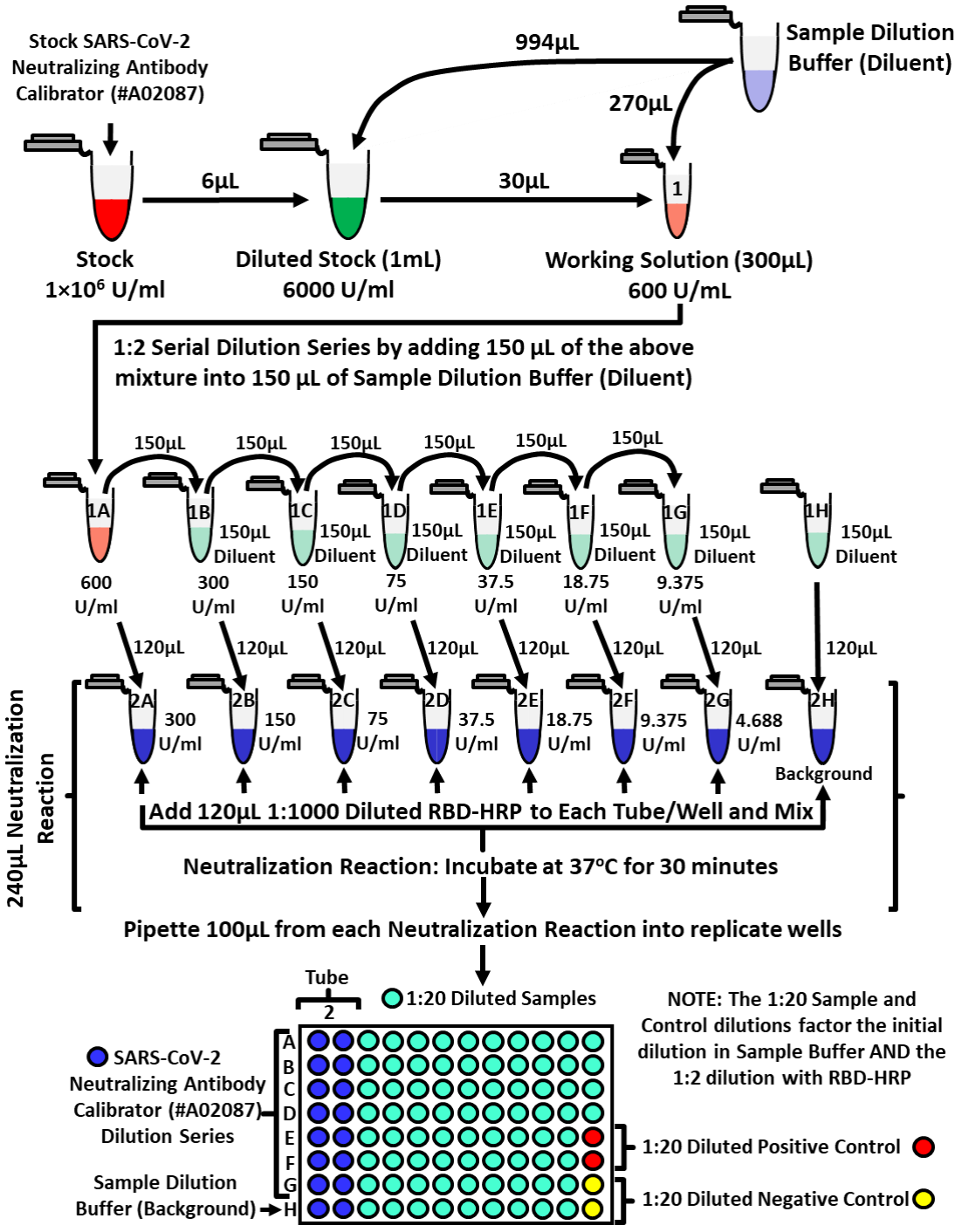


Figure 2. SARS-CoV-2 Neutralizing Antibody (NAb) Calibrator (GenScript #A02087), calibration curve and plating schematic. The neutralizing antibody calibrator and all dilutions should be stored at -20°C.

Samples, calibrators, controls and ACE2-coated capture preparation

1. All positive/negative controls, calibrators and samples should be added in duplicate in a 96-well PCR 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.

Neutralization Reaction Mixtures (Calibration Curve, Samples and Controls)

1. Prepare eight, 1.5ml Eppendorf tubes labelled alphanumerically from "2A" to "2H" consecutively. Transfer 120µL from each of the Calibration Curve dilution tubes ("1A" to "1H") to the corresponding tubes "2A" to "2H" according to Figure 2 above. Alternatively, 120µL of the diluted calibrators can be added directly into the first column of a 96-well PCR "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 PCR plate) and mix with up and down pipetting two times. This will result in 240µL of **Neutralization Reaction** solution for each calibration curve dilution (2A through 2G) and the associated "Background" (2H) (or columns A1 to H1 in a PCR plate). Also, combine 60µL of the 1:10 diluted samples, positive and negative controls with 60µL of the 1:1000 diluted RBD-HRP solution (see "**Reagent Preparation"** subsection above) and mix with up and down pipetting two times. The neutralization reactions for the diluted samples, controls and calibrators

can be prepared in a 96-well, PCR plate to streamline the pipetting and minimize the time for the downstream steps.

3. Incubate the mixtures at 37°C for 30 minutes.

Interaction of Free RBD-HRP with ACE2

1. Add 100µL of the RBD-HRP **Neutralization Reaction Mixtures** (Figure 2) to the corresponding wells of the ACE2-coated assay microtiter plate. Incubate at 37°C for 15 minutes.
2. 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.
3. Pat the plate on paper towel to remove residual liquid in the wells after washing steps.

Substrate Reaction and Absorbance Measurement

1. Add 100µL of TMB Solution to each well and incubate the plate in dark at 25°C for 15 minutes.
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 microtiter plate reader at 450nm immediately.

XI. 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 for manual manipulations should be followed:

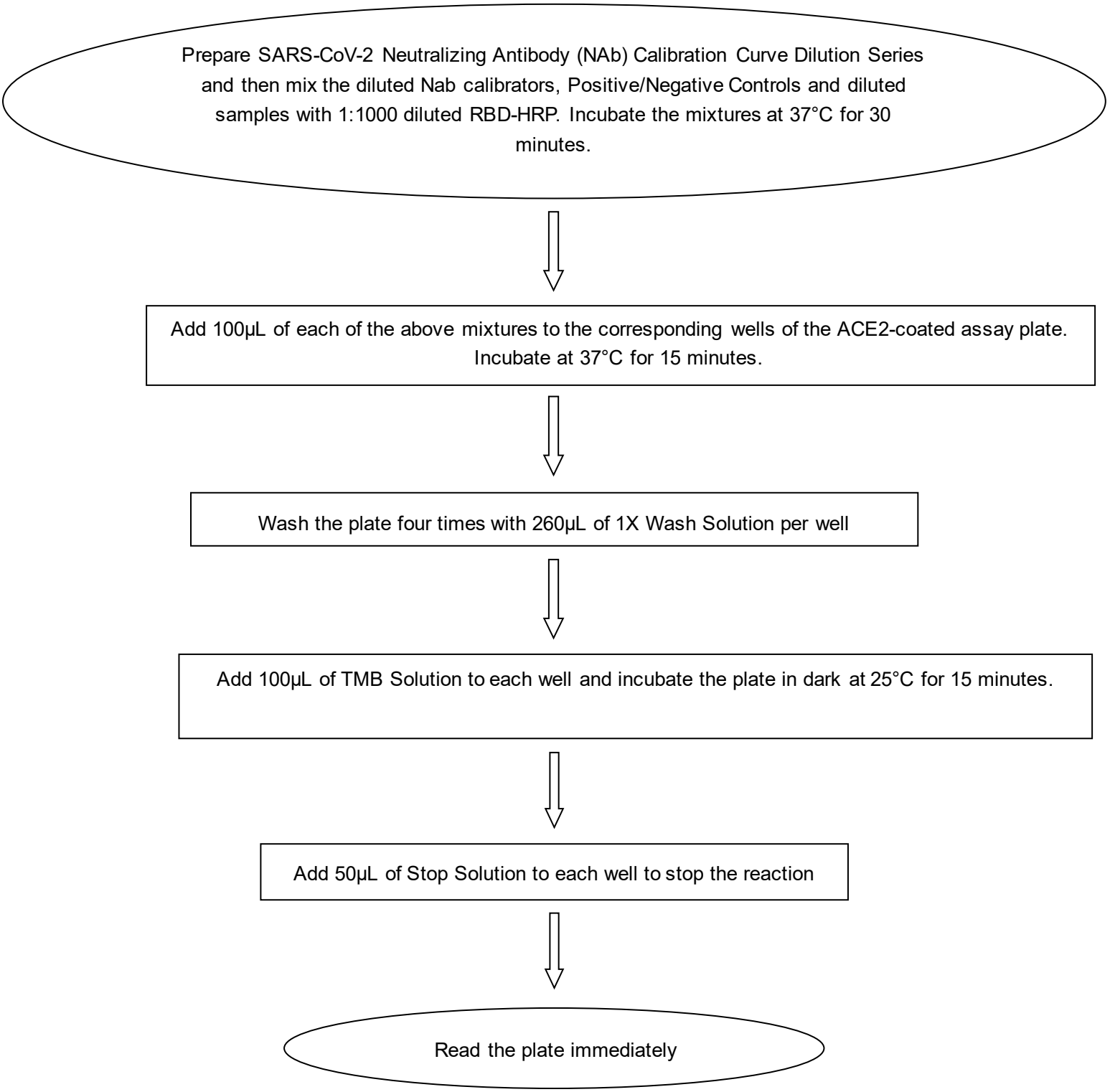
1. Pre-dilute the samples, controls and the SARS-CoV-2 Neutralizing Antibody Calibration curve (Figure 2) into a 96-well PCR plate (Sample Plate).
2. Cover the plate and mix using a standard plate shaker OR by up and down pipetting in the sample plate at least three times. If required perform a quick 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, PCR 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. Incubate the neutralization reaction mixtures at 37°C for 30 minutes. The preparation of the Neutralization Reaction Plate should take less than 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 37°C.
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 calibration 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. The preparation of the ACE2-coated Assay 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 at 37°C.
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. Add 100μL of TMB Solution to each well and incubate the plate in dark at 25°C for 15 minutes. The addition of TMB should not take 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



XII. 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

XIII. INTERPRETATION OF RESULTS (% Signal Inhibition Calculation and Result Interpretation)

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.

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

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

Interpretation of Results:

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. Report as: "NOT DETECTED"
X ⩾ 30%	Positive	X < 47	Positive	Neutralizing antibodies to SARS-CoV-2 are detected. Report as: "DETECTED, <47 Units/mL".
		47 ⩽ X ⩽ 185	Positive	Neutralizing antibodies to SARS-CoV-2 are detected. Report as: "DETECTED, [Add Result VALUE] Units/mL"
		> 185	Positive	Neutralizing antibodies to SARS-CoV-2 are detected. Report as: "DETECTED, > 185 Units/mL"

*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.

**Numerical result in Units/mL are reported only for positive samples that have % Signal Inhibition values of 30% or more. The % Signal Inhibition should not be reported outside of the laboratory.

The clinical applicability of detection or correlation with neutralizing activity for total antibodies to the SARS-CoV-2 receptor binding domain (RBD) at 50% and 90% viral neutralization is currently unknown and results cannot be interpreted as an indication of degree of immunity or protection from infection. Because SARS-CoV-2 neutralizing antibody assays are not standardized, and the performance characteristics of each SARS-CoV-2 neutralizing antibody test is uniquely established, results from different SARS-COV-2 neutralizing antibody assays are not comparable.

XIV. CALCULATION OF SEMI-QUANTITATIVE RESULTS

STEP 1: Calculation of % Signal Inhibition for all samples. Calculate the % signal inhibition for each sample according to section **XIII. INTERPRETATION OF RESULTS (% Signal Inhibition Calculation and Cutoff Values)** above.

STEP 2: Generate the SARS-CoV-2 Neutralizing Antibody (NAb) Calibration Curve. Tabulate then plot the data generated from the NAb calibration curve with Average OD450 on the X-Axis and Concentration (U/mL) on the Y-Axis (Figure 3A). The Concentrations (U/mL) are as follows: 300 U/mL, 150 U/mL, 75 U/mL, 37.5 U/mL, 18.75 U/mL, 9.375 U/mL and 4.688U/mL. To generate the calibration curve, it is recommended to plot the dilution series using a 4 Parametric Logarithmic (4PL) model with a statistical software package. Identify the analytical measuring interval (AMI), according to the **Interpretation of Results Table in Section XIII** (Figure 3B – red dotted lines). The lower and upper limits of the analytical measuring interval are 47 Units/mL and 185 Units/mL respectively.

STEP 3: Use the obtained sample OD450 readings to interpolate the Concentration (U/mL) of each analyzed sample with the Calibration Curve generated in Step 2. The assay cutoff for the cPass SARS-CoV-2 Neutralization Antibody Detection Kit is 30% inhibition, so any qualitative results below 30% inhibition cannot be semi-quantitatively analyzed. For samples with at least 30% inhibition, the U/mL should be calculated using the Calibration Curve generated in Step 2. The U/mL numerical results should be calculated for positive samples within the analytical measuring Interval, according to the **Interpretation of Results Table in Section XIII**.

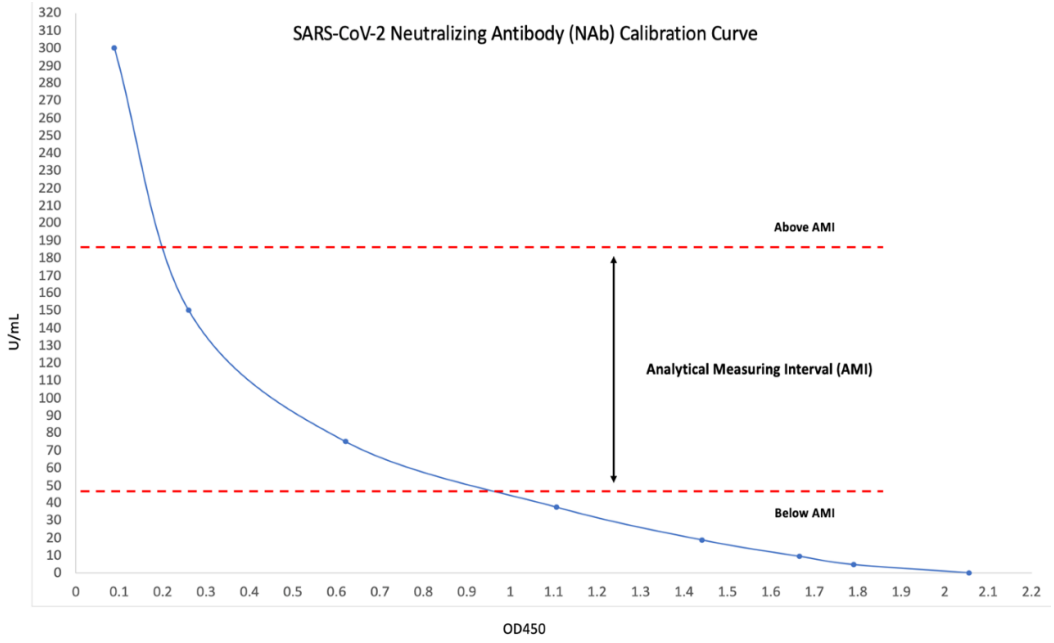
STEP 4: Data Reporting. Refer to the **Interpretation of Results Table in Section XIII** above**.**

Figure 3. Example of Semi-quantitative Result calculation in U/mL **A.** Tabulate the average OD450 values from the SARS-CoV-2 Neutralizing Antibody (NAb) Calibration Curve with the associated concentrations and calculated % inhibition values. **B.** Plot the calibration curve of OD450 versus Concentration (Units/mL) and define the analytical measuring interval (AMI), according to the Interpretation of Results Table in Section XIII.

A.

Concentration (U/mL)	Average Absorbance (OD450)
300	0.0885
150	0.2595
75	0.6205
37.5	1.106
18.75	1.441
9.375	1.6475
4.6875	1.7905
Background	2.056

B.



XV. LIMITATIONS OF THE PROCEDURE

- This test is designed for both qualitative and semi-quantitative detection of SARS-CoV-2 neutralizing antibodies.
- To be used only under the conditions of the FDA Emergency Use Authorization.
- 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.
- 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.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.
- It is unknown at this time if the presence of antibodies to SARS-CoV-2 confers immunity to infection.
- The performance of this test has not been established in individuals that have received a COVID-19 vaccine. The clinical significance of a positive or negative antibody result following COVID-19 vaccination has not been established, and the result from this test should not be interpreted as an indication or degree of protection from infection after vaccination.
- The PPA performance of this test was established based on the evaluation of clinical specimens collected between March and November of 2020 in the US.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens collected in the US from March 2020 to November 2020. Clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- The cPass SARS-CoV-2 Neutralization Antibody Detection Kit is known to cross-react with SARS-CoV-1 neutralizing antibodies.
- This test should not be used for blood donor screening.

XVI. CONDITIONS OF AUTHORIZATIONS FOR THE LABORATORIES:

The cPass SARS-CoV-2 Neutralization Antibody Detection Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

Authorized laboratories using the cPass SARS-CoV-2 Neutralization Antibody Detection Kit ("your product" in the conditions below), must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

A. Authorized laboratories* using your product must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using your product must use your product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

C. Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories must collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Nanjing Genscript Diagnostics Technology Co. Ltd. (at qa@genscript.com) any suspected occurrence of false positive or

false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

F. All laboratory personnel using your product must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in and be familiar with the interpretation of results of the product.

G. Nanjing GenScript Diagnostics Technology Co Ltd, authorized distributors, and authorized laboratories using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

* The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

XVII. 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.

XVIII. 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: $PRNT_{50}$ Result Interpretation:

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: $PRNT_{90}$ Result Interpretation:

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 47 $PRNT_{50}$ 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

	Analyte Level Categories	Titers Sub-Categories	$PRNT_{50}$ Dilution Titers		
				Low Titer	High Titer
			Target Not Detected < 1:20	1:40-1:320	1:640-5120
cPass SARS-CoV-2 Neutralization Antibody Detection Kit interval of numerical values in U/mL	Negative	Negative	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%		

XIX. 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	Mean (Units/mL)	Repeatability		Between Run		Between Day		Within Lab Precision	
			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 – Strong Positive	24	187.396	4.268	2.3%	6.941	3.7%	8.000	4.3%	11.419	6.1%
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%

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 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

XXI. REFERENCES

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DULUTH, MN 55802 USA; 2015.

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E-mail: qa@genscript.com

Version 1.0
Update: DRAFT

SARS-CoV-2 Neutralizing Antibody Calibrator

Instruction for Use

Ref: A02087

For *In Vitro* Diagnostic Use Only

For FDA Emergency Use Authorization Only

For Use with the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (L00847)

For Prescription Use Only

GenScript USA/ Nanjing GenScript Diagnostics Technology Co., Ltd. E-mail: qa@genscript.com

Contents

I. PRODUCT NAME

SARS-CoV-2 Neutralizing Antibody Calibrator

II. PACKING SPECIFICATION

Calibrator Stock 20 μl /bottle (1,000,000 U/mL)

III. INTENDED USE

The SARS-CoV-2 Neutralizing Antibody Calibrator is intended to be used to calibrate the GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit for the qualitative and semi-quantitative direct detection of total neutralizing antibodies to SARS-CoV-2 in human serum and K2-EDTA plasma.

IV. PRINCIPLE

The calibration of a semi-quantitative determination is a process of testing a sample with a known analyte concentration (for example, a measured calibrator) as in a patient sample to calculate its response value. The mathematical relationship between the measured response value and the known analyte concentration can be used to establish a calibration curve. This mathematical relationship, or calibration curve, is used to convert the measured value of the OD (Optical Density value) of the patient sample into a specific semi-quantitative or quantitative analyte concentration.

V. CALIBRATOR CONTENTS

Component	content
SARS-CoV-2 Neutralizing Antibody Calibrator	1 vial (20 µl), containing SARS-CoV-2 neutralizing monoclonal antibody, Phosphate buffer with 2% BSA, 0.1% Proclin-300. Concentration is 1,000,000 U/ml.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

- cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (L00847)
- 10µL, 200µL and 1000µL precision pipettes
- 10µL, 200µL and 1000µL pipette tips

VII. STORAGE CONDITION AND EXPIRE DATE

The unopened calibrator is stable if stored at -20°C, until expiration date and the opened kit is stable for up to 1 month from the date of opening at 2 to 8°C.

VIII. Calibrator Preparation

1. **Calibrator Handling** The Calibrator must be taken out from -20°C and returned to room temperature before use (20 to 25°C). Store all reagents in refrigerator promptly after use.
2. The Calibrator should be vortexed before use.
3. For calibration curve generation and results calculation refer to the IFU of cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit.

▪ Calibrator Dilution

Stock SARS-CoV-2 Neutralizing Antibody Calibrator: The SARS-CoV-2 Neutralizing Antibody (NAb) Calibrator is supplied in a **Stock** solution at a concentration of 1×10^6 Units (U) per mL (U/mL) (Figure 1). Produce a **Diluted Stock** solution of 6000U/mL by mixing 6µL of the **Stock** with

994µL of the kit supplied Sample Dilution Buffer. Each 30µL of the **Diluted Stock** is enough to run the NAb dilution series in duplicate on each plate. Store the **Diluted Stock** of NAb in aliquots frozen at -20°C.

SARS-CoV-2 Neutralizing Antibody Calibrator Working Solution: Dilute the 6000 U/mL **Diluted Stock** (see Step 4 in Reagent Preparation section of the cPass SARS-CoV-2 Neutralization Antibody Detection Kit IFU) 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 1). Calibrator dilutions should be tested in duplicate.

SARS-CoV-2 Neutralizing Antibody Calibration Curve Preparation (see Figure 1)

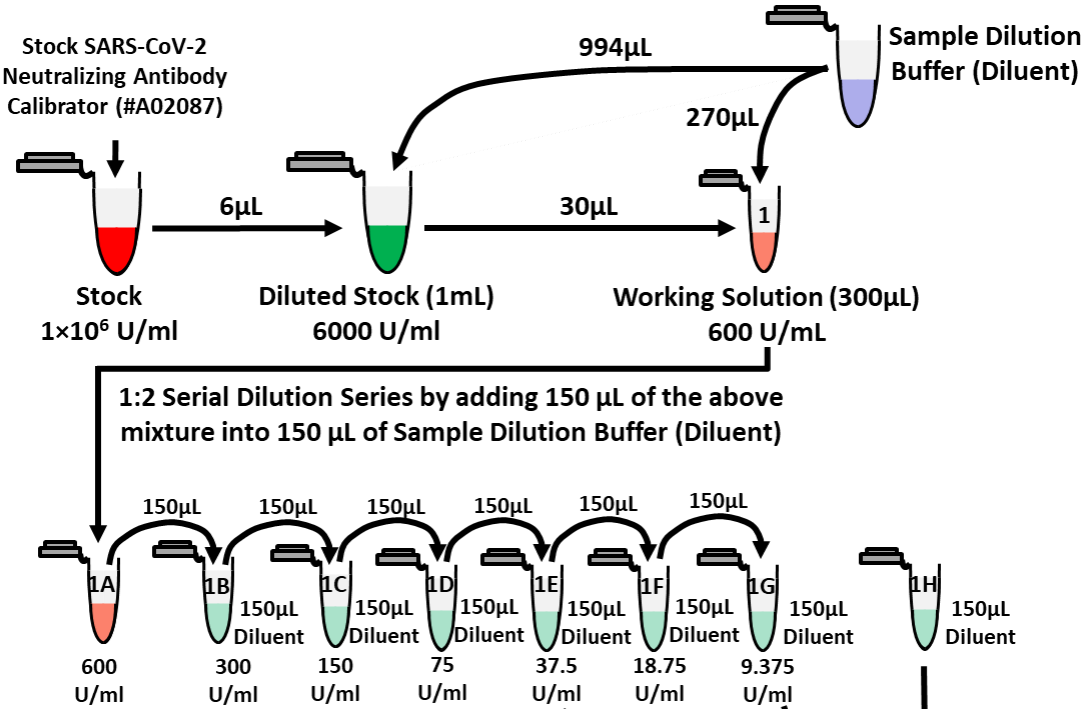
The calibrators can be diluted into a 96-well PCR plate containing the diluted samples, controls and calibrators to streamline the pipetting and minimize the time for the downstream steps. The calibration curve from the **neutralizing antibody calibrator working solution** (described above) is prepared according to the steps below as depicted in Figure 1:

1. The 300µL, **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 1 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.

Figure 1: SARS-CoV-2 Neutralizing Antibody Calibrator dilution schematic



IX. LIMITATIONS OF THE PROCEDURE

1. The user of this kit is advised to carefully read and understand the package insert.
2. If there are signs of microbial contamination or significant turbidity in the reagent, it should be discarded.
3. To only be used with the cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (L00847) under FDA Emergency Use Authorization.

X. Warnings

1. For Prescription and *In Vitro* Diagnostic Use only
2. For Use under an Emergency Use Authorization Only;
3. This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories;
4. This product is for use with a test authorized only for detecting the presence of total neutralizing antibodies to SARS-CoV-2, not for any other viruses or pathogens; and
5. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

XI. PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Only to be use with the cPass SARS-CoV-2 Neutralization Antibody Detection Kit.
3. Do not use the calibrator if there is any visible damage or deviation in physical appearances of component.
4. Do not mix components from different SARS-CoV-2 Neutralizing Antibody Calibrator lots.
5. Do not use calibrator beyond the stated expiration date.
6. The Calibrator must be at room temperature (20 to 25°C) before running assay.

7. Remove only the volume of calibrator that is needed. Do not pour calibrator back into vials as calibrator contamination may occur.
8. Before opening the calibrator, tap the vial or quick spin to ensure that all liquid is at the bottom of the vial.
9. Avoid bubble formation.
10. Decontaminate and dispose of potentially contaminated materials in accordance with local, state, and federal regulations.

XII. REFERENCES

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

XIII. INSTRUCTION APPROVAL AND REVISION DATE

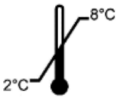
Approval Date:

Revision Date:

Date of Issue:

XIV. INDEX OF CE SYMBOLS

IVD	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	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

XV. TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

GenScript USA Inc.

860 Centennial Ave.
Piscataway, NJ 08854
Technical Support +1 732-885-9188

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: qa@genscript.com