# PhoenixDx® SARS-CoV-2 Neutralizing Antibody Test Kit (ELISA)

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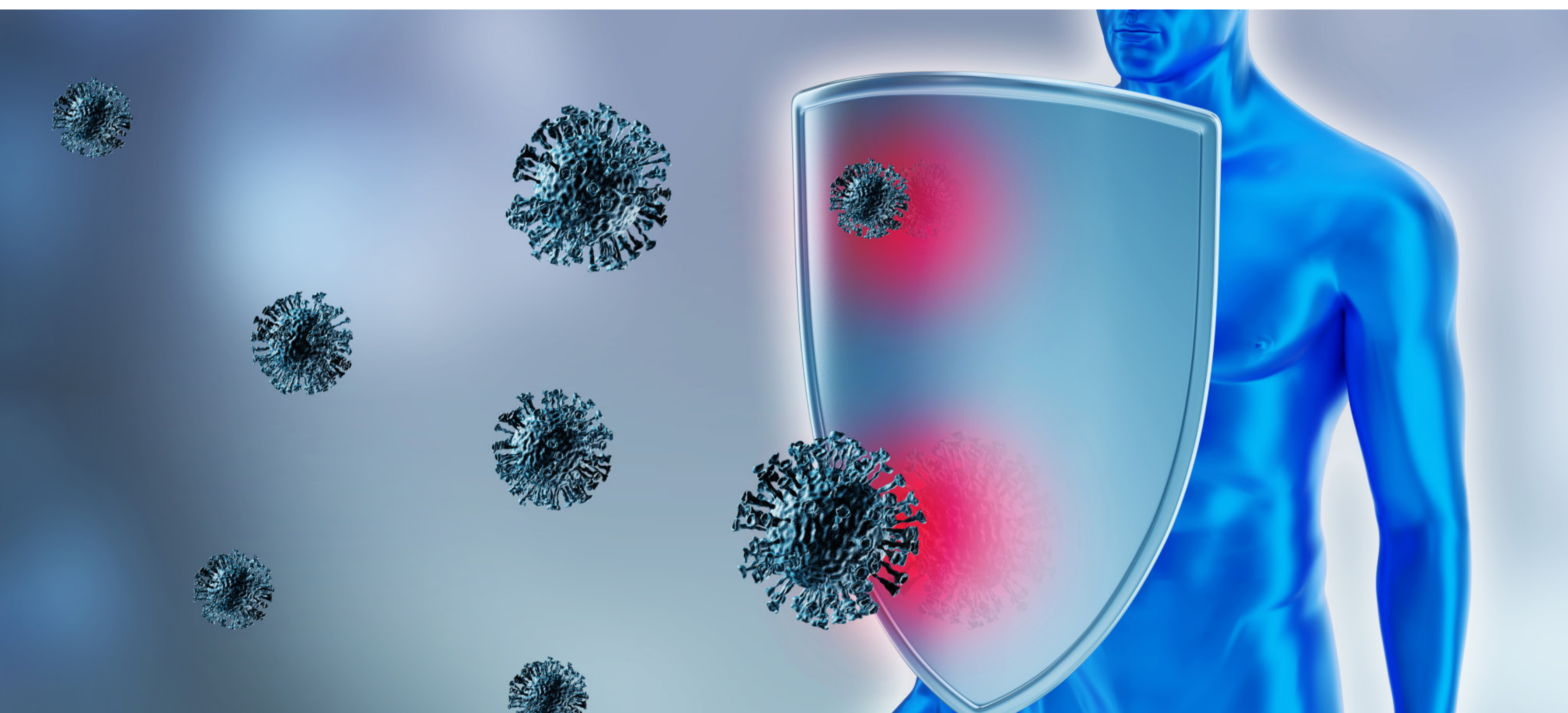
PCCSKU18005

Instructions for Use, v 1.0 04/2021

For medical professional use only.

Please read this manual carefully before operation to ensure proper use.



## Intended Use

The PhoenixDx® SARS-CoV-2 Neutralizing Antibody Test Kit (ELISA) is used for the in vitro qualitative detection of neutralizing antibodies (nAb) against RBD of Novel Coronavirus (SARS-CoV-2) S1 protein in human serum or plasma. A neutralizing antibody can block the protein interaction between RBD and ACE2 thus blocking the virus from entering human cells as SARS-CoV-2 relies on human cell surface protein ACE2 as a cell entry receptor. Production of neutralizing antibodies is stimulated by the envelope or the capsid-antigen of the virus. Only a part of all produced anti-SARS-CoV-2 antibodies are neutralizing antibodies.

The Coronavirus family is divided into four genera, α, β, γ and δ. The α and β genera of coronaviruses can infect mammals and humans. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) belongs to novel coronaviruses of the genus β. It is round or oval with a diameter of 60 ~ 140 nm and appears crown-like under the electron microscope. SARS-CoV-2 contains four major structural proteins: Spike protein (S protein), Nucleocapsid (N protein), Membrane protein (M protein), and Envelope protein (E protein). The S protein has two subunits, S1 and S2, with the receptor binding site (RBD) being located on the S1 subunit. When SARS-CoV-2 invades the body, it will stimulate the body to produce a protective neutralizing antibody, which recognizes the surface protein of the virus and blocks its binding to the ACE2 receptor on the cell surface. The presence and quantity of neutralizing antibodies is an important index of vaccine immunity protection effect and an important basis for vaccine evaluation and quality control.

PhoenixDx® SARS-CoV-2 Neutralizing Antibody Test Kit (ELISA) cannot be used to diagnose acute SARS-CoV-2 infection. Right now, it is unknown for how long antibodies persist after infection and if the detection of neutralizing antibodies proves protective immunity.
Negative results with PhoenixDx® SARS-CoV-2 Neutralizing Antibody Test Kit (ELISA) do not exclude acute SARS-CoV-2 infection. If an acute infection is suspected, direct testing for SARS-CoV-2 with suitable means is necessary.

## Test Principle

The PhoenixDx® SARS-CoV-2 Neutralizing Antibody Test Kit (ELISA) is a competitive ELISA assay. Recombinant human ACE2 receptor protein (hACE2) was used to coat microplates, Horseradish peroxidase-labelled RBD protein (HRP-RBD) is used to prepare the enzyme conjugate.

The first step in the reaction is to pre-incubate samples with HRP-RBD outside the microplate and then transfer them into the hACE2-coated plate. HRP-RBD without binding neutralizing antibodies would bind to hACE2, presence of neutralizing antibodies would block HRP-RBD from binding to hACE2. After adding a chromogenic substrate solution, the activity of HRP generates a non-ferrous product that can be quantified by measuring absorbance at 450 nm. The absorbance is inversely proportional to the effective amount of anti-RBD neutralizing antibody (nAb) in the sample. High absorbance values indicate low amounts or absence of a neutralizing antibodies.

## Components

| Component | Number / Volume |
| --- | --- |
| Microtiter plate: 12 strips coated with hACE2, 8 wells/strip | 12 x 8 wells, 96 wells/plate |
| Enzyme conjugate: HRP conjugated RBD, 1000X | 15 µl |
| Enzyme conjugate diluent | 10 ml |
| Wash Buffer 20X | 15 ml |
| Sample Diluent | 10 ml |
| Positive Control | 50 µl |
| Negative Control | 50 µl |
| TMB Solution | 10 ml |
| Stop Solution: 0.5 M sulfuric acid, direct use | 10 ml |

Equipment and reagents required but not provided in the kit:

- Adjustable pipettes and suitable tips (multichannel pipettes for high sample throughput)
- Single or dual wavelength microplate reader with 450 nm filter
- Microplate Washer
- Deionized or distilled water
- Thermostatic incubator (37°C)
- Absorbent paper
- Tubes / bottles to prepare and store the prepared reagents
- Timer
- Centrifuge
- Storage options for reagents and sample material

## STORAGE CONDITIONS AND VALIDITY PERIOD

Store the kits at 2-8°C to prevent freezing. Avoid direct exposure to strong light. The validity period is 6 months. The kit can be stored at 2-8°C for 1 month after opening. See label for production date and expiration date.

## SAMPLE COLLECTION AND PREPARATION

When working with serum or plasma sample, ***always consider the sample material as potentially infectious***. Treat and dispose of accordingly.
The NCCLS (National Committee for Clinical Laboratory Standards) provides recommendations for handling and storing serum and plasma specimens (Approved Standard-Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).

***Pre-treatment after sample collection***

After collection of the sample, let it sit for 30 min, centrifuge at 1000 g for 10 min to remove precipitate and suspended matter. Use the sample on the same day. If the ELISA test cannot be done on the same day, preserve the sample: serum/plasma samples can be stored for 7 days at 2-8°C, for 6 months at -20°C or below. Avoid heating and repeated freeze/thaw cycles.

# Precautions

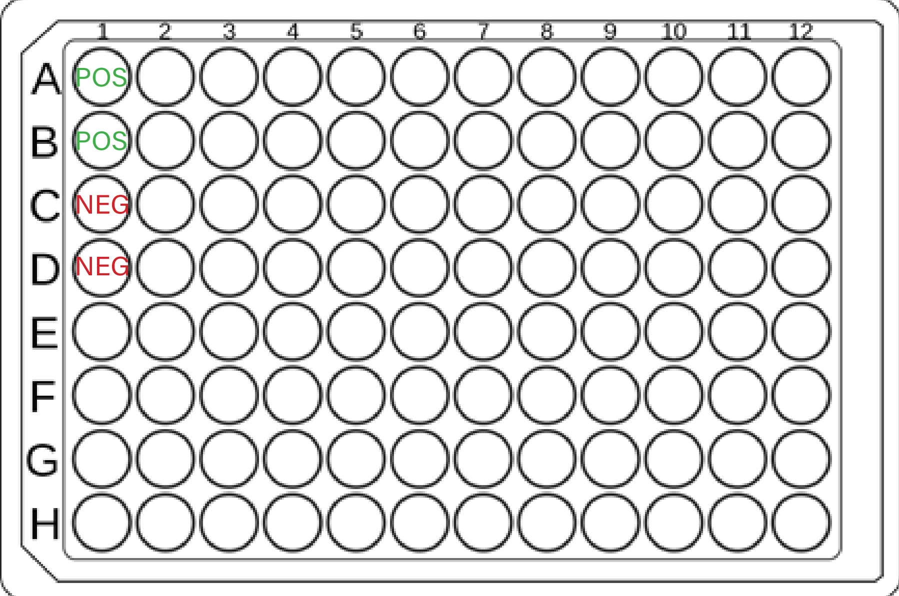
- Please read the instructions carefully before operation and carry out the experiment in strict accordance with the kit instructions.
- Avoid conducting experiments in harsh environment (such as environment containing high concentration of corrosive gas and dust such as 84 disinfectant, sodium hypochlorite, acid and alkali or acetaldehyde). Laboratory disinfection should be carried out after the experiment.
- Check the seal of the aluminium foil bag before use. If the seal is damaged, do not use the kit.
- The sample in the well should be mixed without foaming.
- Do not mix components from different batches. Do not mix with components from other manufacturers.
- Do not let wells dry out while testing; add reagents immediately after washing steps.
- Wash the plate thoroughly but be careful when adding liquid to the wells to avoid spillage.
- Disposable gloves should be worn when handling reagents and samples, hands should be washed after handling. All samples and used kit components should be treated as potentially infectious and discarded in accordance with local and national guidelines and regulations.
- Samples should be tested in a suitable laboratory. All samples and materials during testing are to be considered potentially infectious and treated accordingly.
- The kit should be used within the validity period.

# Test Method

## Experiment Preparation

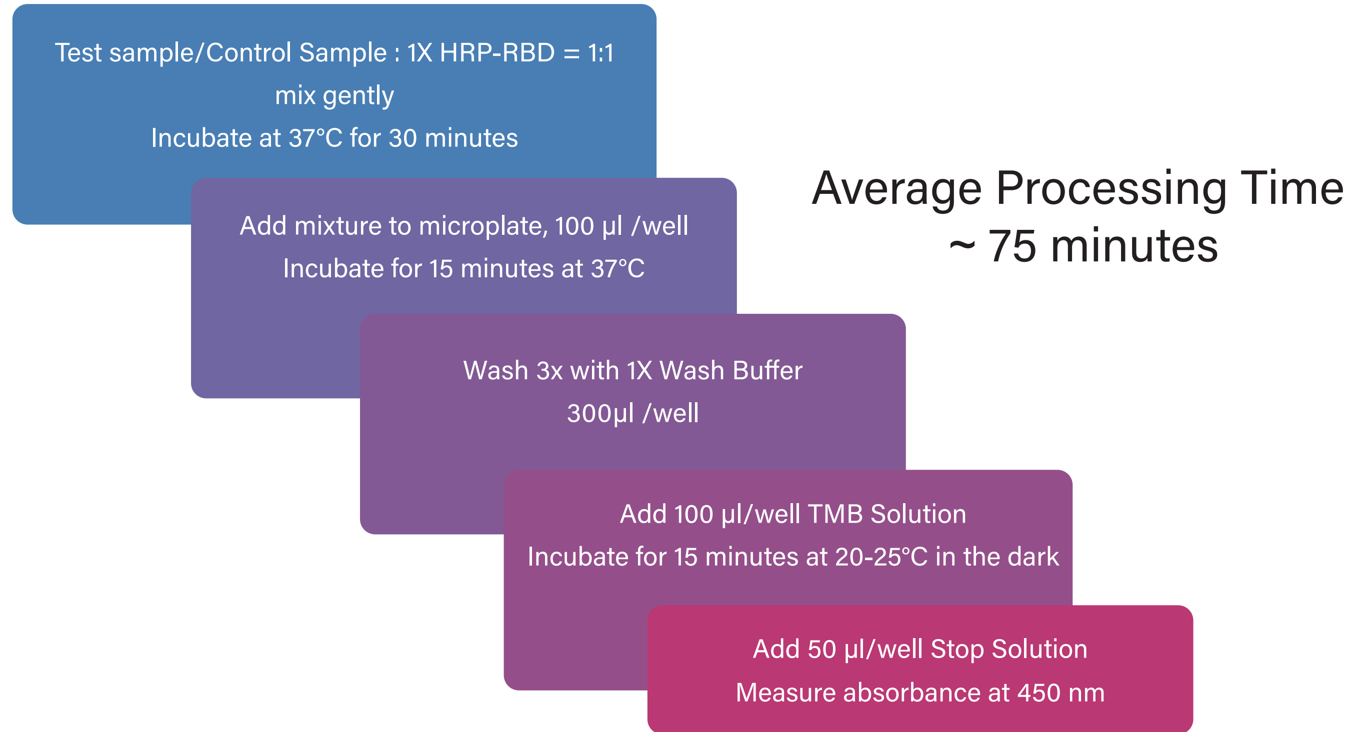
1. It is recommended that all Positive Controls, Negative Controls, and samples should be prepared in duplicates.
2. Install sufficient ELISA strips for all samples to be tested into the plate frame. Make sure the strips are tightly snapped into the plate frame.
3. Equilibrate the kit components to be used and the sample to be tested to room temperature (20-25°C) and mix gently but thoroughly.
4. Preparation of 1X HRP-RBD: 10 µl 1000X HRP-RBD is added into 10 ml enzyme conjugate diluent and mixed gently but thoroughly for later use.
5. Preparation of 1X Wash Buffer: Add 15 ml of 20X Wash Buffer into 285 ml of deionized or distilled water and mix gently but thoroughly.
6. Set the thermostatic incubator to 37°C, use only when the temperature has stabilized.
7. Dilution of sample and reference substance: Dilute the sample to be tested, positive control and negative control with sample diluent at a rate of 1:9 (e.g. 10 µl sample + 90 µl sample diluent) and vortex vigorously.

## Pipetting Scheme




## Experiment Operation

1. In separate tube, mix and dilute the samples/references with 1X HRP-RBD 1:1 (e.g. 60 µl sample + 60 µl 1X HRP-RBD) and incubate at 37°C for 30 minutes.
2. Add 100 µl of the sample mixture, positive control mixture and negative control mixture to the designated wells in the microplate.
3. Seal the microplate and incubate at 37°C for 15 minutes.
4. Remove the supernatant and wash each well 3 times with 1X Wash Buffer (300 µl/well). Gently tap the plate on a paper towel to remove residual Wash Buffer.
5. Add 100 µl TMB substrate solution to each well of the microplate, seal freshly and incubate at room temperature (20-25°C) for 15 minutes in the dark (start timing after addition of TMB substrate solution to the first well).
6. Carefully remove the sealing film and add 50 µl Stop Solution to each well to terminate the reaction.
7. After termination of the reaction, read the absorbance value at 450 nm in a suitable microplate reader. The substrate reaction time is determined by the temperature, the ideal reaction temperature is 25°C. If the temperature is lower than 25°C, extend the reaction time appropriately.




## Results

Negative control and positive control must be included for each test. The expected OD450 of the positive control is < 0.3 and the expected OD450 of the negative control is > 1.0. If the OD450 value of any control does not meet the requirements, this test is invalid and needs to be tested again.

The OD450 of negative control, positive control and sample in a single test was used to calculate the antibody inhibition rate in the serum of the sample to be tested. The OD450 of positive control substance was only used to evaluate the validity of the test. The neutralizing antibody inhibition rate of the sample to be tested is calculated as follows:

$$\text{Inhibition ratio} = \left(1 - \frac{\text{Sample OD450}}{\text{Negative control OD450 mean}}\right) \times 100\%$$

***Result Interpretation***

Cut Off Values should be determined by the user, using the WHO Reference Panel (NIBSC code: 20/136) or similar.

## Limitations of Test Method

1. If test results are inconclusive, re-measure the samples.
2. Due to methodological or immunospecific reasons, different results can be obtained by using reagents from different manufacturers on the same sample, so the data obtained from different reagents should not be directly compared with each other. To avoid flawed interpretation of results, it is recommended that laboratories specify the characteristics of the reagents used in the test report.
3. In serial monitoring, if the reagent type is changed, additional continuous laboratory tests and parallel comparisons with the original reagent results should be performed to re-establish the baseline value.

4. PhoenixDx® SARS-CoV-2 Neutralizing Antibody Test Kit (ELISA) cannot be used to diagnose acute SARS-CoV-2 infection. Right now, it is unknown for how long antibodies persist after infection and if the detection of neutralizing antibodies proves protective immunity.
5. Depending on individual factors, a positive result may not indicate previous SARS-CoV-2 infection. Consider additional information including clinical history and local disease prevalence, in assessing the need for a second but different serology test to confirm an immune response.
6. Until now it is unknown if the presence of antibodies to SARS-CoV-2 confers immunity to re-infection.

## Product Performance

1. Negative reference coincidence rate: Negative reference samples (N1-N10) were used for testing. The conformance rate should be 10/10.
2. Positive reference coincidence rate: the positive reference of the enterprise (P1-P5) is taken as the sample for testing. The conformity rate should be 5/5.
3. LOD reference: S1-S3 are positive, S4 and S5 can be positive or negative.
4. Repeatability: coefficient of variation not greater than 12%.
5. Inter-batch difference: coefficient of variation not greater than 15%.
6. Cross-reactivity: The following pathogenic microorganisms were tested. Test results were negative, indicating that there is no cross-reaction with the following pathogenic microorganisms:

- Endemic human coronaviruses (HKU1, OC43, NL63 and 229E)
- Seasonal H1N1 influenza virus
- H3N2, H5N1, H7N9
- Influenza B Yamagata
- Influenza B Victoria
- Respiratory syncytial virus
- Rhinovirus A, B, C groups
- Adenovirus type 1, 2, 3, 4, 5, 7, 55
- Enterovirus A, B, C, D groups
- Rotavirus, Norovirus antibody positive samples

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

7. Interference response

The following interfering substances were tested, and the test results were all negative indicating that there is no interference with the following test substances:

- α-alpha interferon
- Zanamivir
- Ribavirin
- Oseltamivir
- Peramivir
- Lopinavir, Ritonavir
- Abidor
- Levofloxacin
- Azithromycin
- Ceftriaxone
- Meropenem
- Tobramycin

## 8. Clinical Performance

Comparison between the results of PhoenixDx® SARS-CoV-2 Neutralizing Antibody Test Kit (ELISA) and reference method tested on clinical serum as well as plasma samples.

**Serum samples**: Positive coincidence rate is 97.22% (95%CI: 85.83%-99.51%), Negative coincidence rate is 100.00% (95%CI: 95.19%-100.00%), Total coincidence rate is 99.11% (95%CI: 95.12%-99.84%).

**Plasma samples**: Positive coincidence rate is 100.00% (95%CI: 88.30%-100.00%), Negative coincidence rate is 98.41% (95%CI: 91.54%-99.72%), total coincidence rate is 98.91% (95%CI: 94.10%-99.81%).

***Serum Samples***

| Test | | Reference Method* | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| Assessment System | Positive | 35 | 0 | 35 |
| | Negative | 1 | 76 | 77 |
| Total | | 35 | 76 | 112 |

| Statistical Index | Estimated Value | 95% CI | |
|---|---|---|---|
| | | Lower Limit | Upper Limit |
| Positive Coincidence Rate | 97.22% | 85.83% | 99.51% |
| Negative Coincidence Rate | 100% | 95.19% | 100% |
| Total Coincidence Rate | 99.11% | 95.12% | 99.84% |

* Plaque Reduction Neutralization Test (PRNT) validated surrogate virus neutralization test (sVNT).

***Plasma Samples***

| Test | | Reference Method* | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| Assessment System | Positive | 29 | 1 | 30 |
| | Negative | 0 | 62 | 62 |
| Total | | 29 | 63 | 92 |

| Statistical Index | Estimated Value | 95% CI | |
|---|---|---|---|
| | | Lower Limit | Upper Limit |
| Positive Coincidence Rate | 100% | 88.30% | 100% |
| Negative Coincidence Rate | 98.41% | 91.54% | 99.72% |
| Total Coincidence Rate | 98.91% | 94.10% | 99.81% |

* Plaque Reduction Neutralization Test (PRNT) validated surrogate virus neutralization test (sVNT).

## Reference

[1].Chee Wah Tan. et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. Nature https://doi.org/10.1038/s41587-020-0631-z.

## Label Introduce for User

| Symbol | Meaning | Symbol | Meaning |
|---|---|---|---|
| IVD | *In vitro* diagnostic | LOT | Batch Code |
| | Contains sufficient for <n> tests | | Date of manufacture |
| | Manufacturer | | Use by |
| CE | CE marking | | Temperature Limit 2-8°C |
| | Caution | | This way up |
| | Consult instructions for use | | Keep dry |
| | Biological Risks | | Fragile, handle with care |
| REF | Catalogue Number | | Keep away from sunlight |
| | Do not reuse | | |