# COVID-19 Neutralizing Antibody Rapid Test Kit Instructions For Use

## PRODUCT NAME

FRENOVO COVID-19 Neutralizing Antibody Rapid Test Kit

## PACKAGE SPECIFICATION

20 tests/kit

## INTENDED USE

FRENOVO COVID-19 Neutralizing Antibody Rapid Test is a lateral flow immunoassay intended for the qualitative of neutralizing antibodies (NAbs) to COVID-19 that block the interaction between the receptor binding domain (RBD) of the viral spike glycoprotein with the ACE2 cell surface receptor in human serum, plasma, and whole blood specimens. The test is intended use as an aid in identifying individuals with an adaptive immune response to COVID-19, indicating recent or prior infection. FRENOVO COVID-19 Neutralizing Antibody Rapid Test should not be used to diagnose acute COVID-19 infection.

Results are for the detection of total neutralizing antibodies to COVID-19. Antibodies to COVID-19 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. Negative results do not preclude acute COVID-19 infection. If acute infection is suspected, direct testing for COVID-19 is necessary. False positive results may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

## SUMMARY AND PRINCIPLES OF THE PROCEDURE

The novel corona viruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel corona virus are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

COVID-19 has several structural proteins including spike (S), envelope (E), membrane (M) and nucleocapsid (N). The spike protein (S) contains a receptor-binding domain (RBD), which is responsible for recognizing the cell surface receptor, angiotensin converting enzyme-2 (ACE2). It is found that the RBD of the COVID-19 S protein strongly interacts with the human ACE2 receptor leading to endocytosis into the host cells of the deep lung and viral replication.

The scientist has proofed that the neutralization epitope is a specific receptor binding domain of the S protein of the virus spikes, called S-RBD. After Infection with COVID-19, human will initiate immune response, which includes the production of antibodies. Among the total antibodies to the virus, the neutralizing antibodies will bind to the S-RBD and block cellular infiltration and replication of the virus. It is unknown how long it takes for neutralizing antibodies to be produced, and if they are always produced after COVID-19 infection. While individuals infected with COVID-19 develop binding antibodies to the virus, not all of them develop neutralizing antibodies to COVID-19. FRENOVO COVID-19 Neutralizing Antibody Rapid Test is specific to COVID-9 neutralizing antibodies.

FRENOVO COVID-19 Neutralizing Antibody Rapid Test utilizes the sandwich immunoassay to detect the total neutralizing antibodies. When the specimen is added into the sample well of the test cassette, the neutralizing antibodies in the specimen will bind to the gold labeled S-RBD, the antibody-antigen complex goes forward on the membrane. At the T line, the complex will be captured by another recombinant viral S-RBD protein and display as a red line, if neutralizing antibodies are present in the specimen. If no neutralizing antibodies in the specimen or the amount of them is too low, the gold labeled S-RBD will go through at the T line, and no visible red line will display. The Control Line is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working.

## MATERIALS PROVIDED

Each kit contains:

1. test cassettes: 20 pieces test cassettes individually pouched.
2. Wash Buffer Solution: 2.0 ml in dropper bottle.
3. Droppers: 20 pieces droppers of 20 ul.
4. Package insert: 1 piece attached.

## MATERIALS REQUIRED BUT NOT PROVIDED

- Timer or stopwatch.
- Specimen collection containers
- Disposable gloves and/or protective clothing
- Centrifuge(for plasma only)
- Micro-pipette

## WARNINGS

1. Read the package insert completely before using the product. The instructions must be followed carefully as not doing so may result in inaccurate results.
2. The kits for diagnostic use only.
3. Perform test at room temperature.

## PRECAUTIONS

1. FRENOVO COVID-19 Neutralizing Antibody Rapid Test Kit is for professional use only.
2. The package insert instructions must be followed to ensure optimum test performance.
3. The kit is intended for *in vitro* diagnostic use.
4. As with all screening assays, any results should be considered presumptive until confirmatory assays have been performed according to local practice or WHO guidelines.

### Safety Precautions

1. Standard precautions for handling infectious agents should be observed when using this kit.
2. Wear protective clothing such as lab coat, safety glasses and disposable gloves when handling specimens and assay reagents.
3. Wash hands thoroughly after use.
4. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

### Bio safety Precautions

Appropriate bio safety practices should be used when handling specimens and reagents. These precautions include, but are not limited to the following:

1. Do not smoke,eat,drink,apply cosmetics or handle contact lenses in areas in which specimens are handled.
2. Dispose of all specimens, used devices and tubes as though they are capable of transmitting infection. The preferred methods of disposal are by autoclave at 121°C for a minimum of 60 minutes or by incineration. Disposable materials may be incinerated. Liquid waste may be mixed with appropriate chemical disinfectants. A solution of 10% bleach is recommended. Allow 60 minutes for effective decontamination. NOTE: Do not autoclave solutions containing bleach.
3. When disposing of solution, avoid contact with acid to prevent liberation of a toxic gas.
4. All spills should be wiped thoroughly using a suitable disinfectant such as a sodium hypochlorite solution.
5. Use a separate dropper and device for each specimen tested.

### Handling Precautions

1. Do not use if the kit safety seal is absent, damaged or broken.
2. Do not use any device if the pouches have been perforated.

3. Each device is for single use only.
4. Do not mix wash buffer solution/test cassettes from different kit lots.
5. Do not use the kit past the expiration date (this date is printed on the kit box).
6. Adequate lighting is required to read the test results.
7. The result should be read immediately after the end of the 10 minutes incubation time following the addition of specimen and wash buffer solution. Do not read results beyond 15 minutes.

## STORAGE INSTRUCTIONS

1. The kit should be stored between 2-30°C and the shelf life is 24 months.
2. The kit components are stable until the expiration date printed on the outer label, when stored as directed. The kit expiry date is determined by whichever of the components has the shortest expiry date. The kit expiry date is not impacted once the wash buffer solution has been opened. Do not use kit components beyond overall kit expiry date.
3. If stored refrigerated, ensure that the pouched device is brought to room temperature before opening.
4. Do not freeze the kit.

## SAMPLE COLLECTION AND PREPARATION

1. Applicable samples：Whole Blood/Serum/Plasma.
2. Separate serum or plasma from whole blood as soon as possible to avoid hemolysis. Use only clear nonhemolysis specimens.
3. Testing should be performed immediately after the specimens have been collected as soon as possible . Do not leave the specimens at room temperature for prolonged periods.
4. Serum and plasma specimens may be stored at 2~8 ℃ for up to 7 days, for long term storage, serum/plasma specimens should be kept below -20℃. Whole blood collected by venipuncture should be stored at 2~8℃ if the test is to be run within 2 days of collection.
5. Do not freeze whole blood specimens.
6. Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
7. If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiological agents.
8. EDTA K2, Heparin sodium, Citrate sodium and Potassium Oxalate can be used as the anticoagulant for collecting the specimen.

## QUALITY CONTROL

An internal procedural control is included in the test. a colored line appearing in the control line region (C) is an internal valid procedural control, it confirming adequate membrane wicking. Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

## TEST PROCEDURE

**Allow the test cassette, specimen, buffer solution to equilibrate to room temperature (15-30°C) prior to testing.**

1. Remove the test cassette from the sealed pouch and use it within one hour. Place the test cassette on a clean and level surface.

2. For Serum or Plasma Specimens

   To use a dropper: Hold the dropper vertically, draw the specimen and transfer the specimen to the sample well of the test cassette (one drop/approximately 20ul), then squeeze the wash buffer solution bottle, add two drops wash buffer solution inside (approximately 60ul) to the sample well and start the timer. Avoid trapping air bubbles in the sample well.

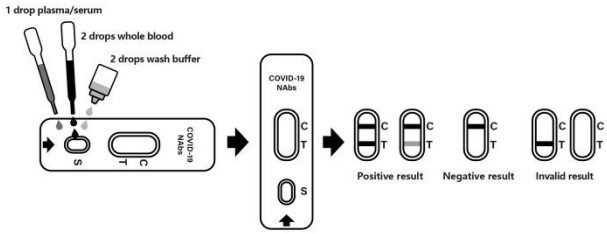
   To use a micro-pipette: Pipette and dispense 20ul of specimen to the sample well of the test cassette, then squeeze the wash buffer solution bottle, add two drops wash buffer solution inside (approximately 60ul) to the sample well and start the timer.

   For Whole Blood Specimens

   To use a dropper: Hold the dropper vertically, draw the specimen and transfer the specimen to the sample well of the test cassette (two drops/approximately 40ul), then squeeze the wash buffer solution bottle, add two drops wash buffer solution inside (approximately 60ul) to the sample well and start the timer. Avoid trapping air bubbles in the sample well.

   To use a micro-pipette: Pipette and dispense 40ul of specimen to the sample well of the test cassette, then squeeze the wash buffer solution bottle, add two drops wash buffer solution inside (approximately 60ul) to the sample well and start the timer.

3. Wait for the colored line(s)to appear. The test result should be read at 10 minutes. Do not interpret the result after 15 minutes.




## INTERPRETATION OF RESULTS

**Negative result:** if there is only a quality control line C, the detection line is colorless, indicating that COVID-19 neutralizing antibody has not been detected and the result is negative.

**Positive result:** if both the quality control line C and the detection line appear, the COVID-19 neutralizing antibodies has been detected and the result is positive.

**Invalid result:** Control line fails to appear. Insufficient buffer volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the procedure with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

**NOTE:** The intensity of the color in the test line region(s) will vary depending on the concentration of COVID-19 neutralizing antibodies in the specimen.

## LIMITATIONS

1. The kit is for in vitro diagnostic use only. The test should be used for the detection of COVID-19 neutralizing antibodies in serum, plasma or whole blood specimens only.
2. In the early stage after vaccine injection, COVID-19 neutralizing antibodies concentrations may be below detectable levels.
3. A positive result may not indicate previous COVID-19 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.
4. Results from immunosuppressed patients should be interpreted with caution.
5. Results from this test should not be used to diagnose or to exclude acute COVID-19 infection

   or to inform infection status.

## PERFORMANCE CHARACTERISTICS

**Sensitivity and Specificity**

Clinical study was performed to compare the results obtained by The kit and PCR. The results indicated that The kit has a high sensitivity and specificity as summarized below:

| Method | | PCR | | |
|---|---|---|---|---|
| COVID-19 Neutralizing Antibody Rapid test | Results | Positive | Negative | Total Results |
| | Positive | 285 | 1 | 286 |
| | Negative | 5 | 99 | 104 |
| Total Results | | 290 | 100 | 390 |

**Summary Results:**

Clinical sensitivity =98.28% (95%CI*96.02%～99.44%)

Clinical specificity = 99.00% (95%CI*94.55%～99.97%)

Accuracy=98.46% (95%CI*96.68%～99.43%)

**Interference Substances**

The following potential interfering substances have been tested using The kit and no interference was observed :

| Substance | Tested Concentration |
|---|---|
| Hemoglobin | 6 mg/ml |
| Bilirubin | 0.4 mg/ml |
| Triglycerides | 15 mg/ml |
| Cholesterol | 4 mg/ml |
| Human Anti-mouse Antibody (HAMA) | 100 IU /ml |
| Rheumatoid Factor | 1500 IU/ml |
| Antinuclear Antibody (ANA) | 1:640 |
| α-interferon | 2 ng/ml |
| Lopinavir | 2 μg/ml |
| Tobramycin | 10 mg/L |
| Ribavirin | 40 mg/L |
| Zanamivir | 140 ng/ml |
| Ritonavir | 50 μg/ml |
| Tramadol | 12 μg/ml |
| Azithromycin | 5 μg/ml |
| Meropenem | 10 mg/ml |
| Oseltamivir | 1000 ng/ml |
| Mupirocin | 10 mg/ml |
| benzocaine | 1.5 mg/ml |
| Peramivir | 20 μg/ml |
| Epinephrine | 500 pmol/L |

**Cross Reaction**

Cross-reactivity of The kit was evaluated using serum samples containing antibodies to other pathogens., which has no effect on the negative and positive test results, and there is no cross-reaction.

| Potential Cross-reactant | Potential Cross-reactant |
|---|---|
| Influenza A virus (H1N1, H3N2 ) | Enterovirus 71 |
| Influenza B virus (Yamagata, Victoria) | Coxsackie virus type A16 |
| Endemic human coronavirus (OC43, 229E) | Varicella zoster virus |
| CMV | Mumps Virus |
| Rubella | Respiratory syncytial virus |
| Toxo | Adenovirus |
| HSV | Chlamydia pneumoniae |
| Coxsackie virus group B | Mycoplasma pneumoniae |
| Epstein-Barr virus | Measles virus |

**INDEX OF SYBOML**

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|---|---|---|---|
| IVD | In vitro diagnostic medical device | | single-use,Please don't reuse it |
| | Use-by date | | Consult instructions for use |
| | Cautions | | Manufacturer |
| 2°C 30°C | Temperature limit | LOT | Batch code |
| | Date of manufacture | | Keep Dry |
| | Avoid overexposure to the sun | | Don't use the product when the package is damaged |
| CE | CE mark | | Biological risks |


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**INSTRUCTION APPROVAL AND REVISION DATE**

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Date of Issue: