

**INTENDED USE**

The 2019-nCoV RBD antibodies are protective antibody produced by the human body after inoculation with 2019-nCoV vaccine or infection with 2019-nCoV.

The test is intended as an aid to assess the adaptive humoral immune response to the 2019-nCoV.

For *in vitro* diagnostic use only. For professional use only.

**SUMMARY**

The 2019-nCoV belong to the  $\beta$  genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible.

The current research has found that the 2019-nCoV binds to their host cell receptor, angiotensin-converting enzyme 2 (ACE2) through RBD from the spike protein (S protein), and undergoes a process of cell membrane fusion. Then the virus enters the cell through endocytosis, replicates and infects the body.

Antibodies against 2019-nCoV with strong neutralizing capacity, especially potent if directed against the RBD, have been identified. Numerous vaccines for COVID-19 are in development, many of which focus on eliciting an immune response to the RBD.

**PRINCIPLE**

2019-nCoV RBD Antibody Rapid Test is based on the principle of double antigen sandwich immunoassay for determination of 2019-nCoV RBD antibodies in human whole blood, serum and plasma specimen.

Add specimens into the sample well to initial a test. When the 2019-nCoV RBD antibodies level in the specimen is at or above the detection limit of the test, the RBD antibodies bound to the colloidal gold labeled 2019-nCoV recombinant antigen and form an immune complexes which are captured by RBD immobilized in the Test Region (T) of the nitrocellulose membrane, and this produces a colored test band that indicates a positive result. When the 2019-nCoV RBD antibodies level is zero or below the detection limit, there is no visible colored band in the Test Region (T) of the device. This indicates a negative result.

To serve as a procedure control, a colored line will appear at the Control Region (C), if the test has been performed properly.

**PRECAUTION**

1. This kit is for *in vitro* diagnostic use only.
2. Sealed pouch contains Desiccant pouch, which is for storage purposes only, and is not used in test procedure.
3. All specimens should be treated as capable of transmitting diseases. Use appropriate precautions in the collection, handling, storage and disposal of testing samples and used kit contents. And follow biosafety level 2 or higher guidelines.
4. Wear appropriate personal protective equipment (e.g. medical gloves, medical mask, goggles and lab coat) when handling the contents of this kit.
5. Proper specimen collection, storage and transport are critical to the performance of this test.
6. Discard after first use. The test cannot be used more than once.

7. Avoid excessively high temperature in the testing environment. Test Cassettes and Detection Buffer stored at low temperature need to be returned to room temperature before opening to avoid moisture absorption.
8. Do not touch the reaction area of test strip.
9. Do not use test kit beyond the expiration date.
10. The Test Cassette should remain in its original sealed pouch until use. Do not use the kit if the pouch is punctured or not well sealed.
11. Do not use damaged or stained detection buffer tube.
12. Testing should be applied by professional trained staff working in certified laboratories or clinics at which the specimen(s) is taken by qualified medical personnel.
13. The test result should be interpreted by the physician along with clinical findings and other laboratory test results.
14. DISPOSAL OF THE DIAGNOSTIC: All specimens and the used kit has the infectious risk. The process of disposing the diagnostic must follow the local infectious disposal law or laboratory regulation.
15. If you have questions or suggestions during the use of this reagent, please contact the manufacturer.

**MATERIAL****Material Provided**

REF	Sealed pouches*	Disposable Droppers	Detection Buffer**	Sterile Lancet***	Alcohol Pad	IFU
W632P0002	2	2	2*200 $\mu$ L	2	2	1
W632P0003	5	5	5*200 $\mu$ L	5	5	1
W632P0004	10	10	10*200 $\mu$ L	10	10	1
W632P0005	20	20	20*200 $\mu$ L	20	20	1
W632P0006	10	10	1*6 mL	/	/	1
W632P0007	20	20	2*6 mL	/	/	1
W632P0008	25	25	25*200 $\mu$ L	25	25	1
W632P0009	40	40	40*200 $\mu$ L	40	40	1
W632P0010	25	25	2*6 mL	/	/	1
W632P0011	40	40	4*6 mL	/	/	1

**Note:**

\*each sealed pouches containing: 1 Test Cassette and 1 Desiccant Pouch

\*\* There are two types of Detection Buffer: buffer in a capsule (200  $\mu$ L/capsule) and buffer in a dropper bottle (6 mL/bottle).

\*\*\* CE information of Sterile Lancet:  MDD 93/42/EEC

**Reactive ingredients of main components**

The test cassette consists of test strip and plastic cassette. The test strip includes: nitrocellulose membrane, sample pad, conjugated pad, absorbent paper and PVC board. Nitrocellulose membrane is coated with RBD, anti-rabbit IgG polyclonal antibodies; Conjugate pad contains 2019-nCoV recombinant antigen and rabbit IgG.

**Note:** To ensure the accuracy of test results, components in different lots cannot be mixed-used.

**Material Required but Not Provided**

1. Specimen Collection Containers
2. Centrifuge (for serum/plasma specimen)
3. Timer
4. Personal protective equipment, such as medical gloves, medical mask, goggles and lab coat.
5. Appropriate biohazard waste container and disinfectants.

**STORAGE AND STABILITY**

1. Store at 2~30°C in the sealed pouch up to the expiration date printed on the package. Do not freeze.

2. The test cassette should be used within 1 hour after taking out from the foil envelope. Buffer solution should be re-capped in time after use.
3. Keep away from sunlight, moisture and heat.
4. Kit contents are stable until the expiration date printed on the outer box.
5. The production date is printed on the outer box.

**SPECIMEN COLLECTION AND PREPARATION**

The test can be performed with fingerstick Whole Blood, venous whole blood, serum and plasma.

**For Fingerstick Whole Blood:**

1. Clean the area to be lanced with an alcohol pad. Allow the finger to dry thoroughly.
2. Use a sterile lancet, puncture the skin just off the center of the finger pad. Apply gentle pressure beside the point of the puncture. Wipe away the first drop of blood. Allow a new drop of blood to form. If blood flow is inadequate, the subject's finger may have to be gently massaged at the finger base to produce a droplet of sufficient volume.
3. Draw 20  $\mu$ L of finger blood with the disposable dropper. Whole blood specimen collected by fingerstick should be tested immediately.

**For Venous Whole Blood:**

1. According to standard venous blood collection procedure, collect a venipuncture whole blood specimen using a blood collection tube with suitable anticoagulant (containing EDTA or Heparin). **Other anticoagulants have not been validated and may give incorrect result.**
2. It is recommended that whole blood specimen is tested at the time of specimen collection. If the specimens are not tested immediately, they may be stored at 2~8°C for up to 3 days. Prior to testing, mix the blood by gentle inversion several times, do not freeze or heat whole blood specimens.

**For Serum and Plasma:**

1. According to standard venous blood collection procedure, collect a venipuncture whole blood specimen using a blood collection tube. If collecting plasma use a blood collection tube containing suitable anticoagulant (containing EDTA or Heparin). **Other anticoagulants have not been validated and may give incorrect result.**
2. Separate the serum/plasma from blood as soon as possible to avoid hemolysis.
3. Test should be performed within 8 hours after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods.
4. If the specimens are not tested within 8 hours, they should be kept at 2~8°C for up to 7 days. For storage more than 7 days, specimens should be kept below -20°C for long time.

**Note:** Bring specimens to room temperature before testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly. Severe hemolytic or heat-inactivated specimens are not recommended.

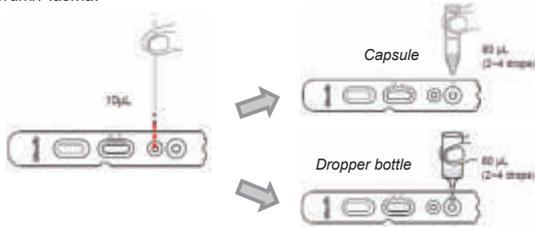
**TEST PROCEDURE**

Please read the instruction for use carefully before performing the test.

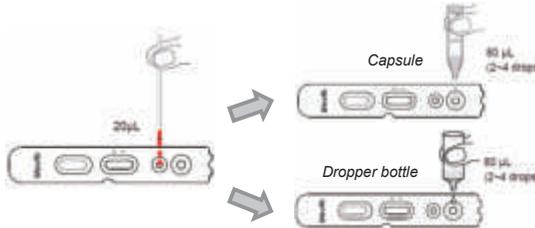
1. Allow the device, buffer and specimen to equilibrate to room temperature prior to testing.
2. Remove a test cassette from the sealed pouch by tearing at the notch and place it on a level surface.
3. Add **10  $\mu$ L** serum/plasma or **20  $\mu$ L (1 drop)** fingerstick/venous whole blood specimen into the sample well (small well) with the disposable dropper, and then add **80  $\mu$ L (2~4 drops)** detection buffer (in a dropper bottle or capsule) into the buffer well (large well) as the below picture shows.
4. As the test begins to work, you will see purple color move across the result window in the center of the test device.
5. Wait for **15 minutes** and read the results.

**Note:** Results after more than 30 minutes may be not accurate and should not be read.

Serum/Plasma:



Fingerstick/Venous Whole Blood:



## RESULT INTERPRETATION

### Positive Result

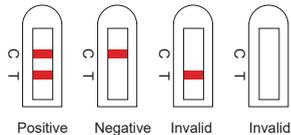
Colored band appear at both test line (T) and control line (C). It indicates that the concentration of the 2019-nCoV RBD antibodies at or above the detection limit of the test.

### Negative Result

Colored band appears only at control line (C). It indicates that the concentration of the 2019-nCoV RBD antibodies is zero or below the detection limit of the test.

### Invalid Result

No visible colored band appear at control line after performing the test. The directions may not have been followed correctly. Pay particular attention to whether the sample volume is sufficient. It is recommended that the specimen be re-tested.



## QUALITY CONTROL

A procedural control is included in the test. A colored line appearing in the control region (C) is considered an internal procedural control. It confirms sufficient liquid volume, adequate membrane wicking and correct procedural technique.

Good laboratory practice recommends the use of the control materials. Users should follow the appropriate federal state, and local guidelines concerning the frequency of assaying external quality control materials.

## LIMITATIONS OF PROCEDURE

1. This reagent is designed to detect RBD antibodies against 2019-nCoV in human whole blood, serum or plasma specimen.
2. The accuracy of the test depends on the specimen collection process. Improper specimen collection, improper specimen storage, or repeated freezing and

thawing of the specimen will affect the test result.

3. This reagent is a qualitative assay. It is not designed to determine the quantitative concentration of 2019-nCoV RBD antibodies. If you need to test the quantitative concentration, please use the relevant professional instruments.
4. A positive result cannot completely rule out the risk of infection with the coronaviruses (2019-nCoV).
5. Limited by the method of antibody test reagents, for negative test results, it is recommended to use other methods for review and confirmation.
6. A negative result of this reagent can be caused by:
  - 1) improper specimen collection, improper specimen transfer or handing, the antibodies titer in the specimen is too low;
  - 2) variations in viral genes may cause changes in antibodies determinants.

## PERFORMANCE CHARACTERISTICS

### A. Sensitivity and Specificity

A total of 238 samples retrospectively collected from 2019-nCoV PCR positive and negative individuals (65 positive and 173 negative) were tested with the 2019-nCoV RBD Antibody Rapid Test.

The sampling date of positive samples were both more than 7 days after diagnosis with PCR.

Reagents		PCR		Total
		Positive	Negative	
2019-nCoV RBD Antibody Rapid Test	Positive	62	1	63
	Negative	3	172	175
Total		65	173	238

Sensitivity: 95.38% (95%CI: 87.29%~98.42%)

Specificity: 99.42% (95%CI: 96.80%~99.90%)

Overall agreement: 98.32% (95%CI: 95.76%~99.34%)

### B. Correlation of test results to serum neutralization capacity

The 2019-nCoV RBD Antibody Rapid Test was compared to The Pseudovirus Neutralization Assay. The following tables show the correlation between NtAb<sub>50</sub>\* of Pseudovirus Neutralization Assay and results of the 2019-nCoV RBD Antibody Rapid Test.

Reagents		Pseudovirus Neutralization Assay			Total
		< 1:20	1:20~1:160	≥1:160	
2019-nCoV RBD Antibody Rapid Test	Negative	20	1	0	21
	Positive	1	6	24	31
Total		21	7	24	52

\*NtAb<sub>50</sub>= Antibody titers resulting in 50% virus neutralization:

Dilution Titer	Result	Test Result Interpretation
< 1:20	Negative	Neutralizing antibodies for 2019-nCoV are not detected at 50% virus neutralization.
1:20~1:160	Low titer	Low neutralizing antibodies for 2019-nCoV are detected at 50% virus neutralization.
>1:160	High titer	High neutralizing antibodies for 2019-nCoV are detected at 50% virus neutralization.

### C. Cross-reactivity

Cross-reactivity of the 2019-nCoV RBD Antibody Rapid Test was evaluated using specimens containing the antibodies listed below. The results showed no cross

reactivity with the following:

Parainfluenza virus antibodies	Treponema pallidum antibodies
Influenza A antibodies	HIV antibodies
Influenza B antibodies	EB virus antibodies
Chlamydia pneumonia antibodies	Measles virus antibodies
Mycoplasma pneumoniae antibodies	Cytomegalovirus antibodies
Adenovirus antibodies	Enterovirus type 71 antibodies
Respiratory syncytial virus antibodies	Mumps antibodies
Hepatitis B surface antibodies	Varicella-zoster virus antibodies
Hepatitis C virus antibodies	

### D. Interference

The test result of 2019-nCoV RBD Antibodies Test were not be interfered with the substance at the following concentration:

Substance	Concentration
Bilirubin	250 µmol/L
Hemoglobin	9 g/L
Triglyceride	15 mmol/L
Rheumatoid factors	80 IU/mL
Antinuclear antibodies (ANA) titer	1:240
Anti-mitochondrial antibodies (AMA)	80 U/mL
HAMA	1000 µg/mL

### E. Hook effect

Within the titer range of clinically positive specimens of 2019-nCoV RBD antibodies, there was no hook effect in the test results of this product.

### F. Precision

1. Within-run Precision was determined by testing separately two positive specimens in 10 times. The agreement rate was 100%.
2. Between-run Precision was determined by testing three different specimens including positive and negative in 3 different lots of test devices. The negative agreement rate and the positive agreement rate were 100%.

## BIBLIOGRAPHY

- [1] Burton, D. R. Antibodies, viruses and vaccines. Nat. Rev. Immunol. 2,706-713 (2002).
- [2] Klasse, P. J. Neutralization of virus infectivity by antibodies: old problems in new perspectives. Adv. Biol. <https://doi.org/10.1155/2014/157895> (2014)

## INDEX OF SYMBOL

<b>IVD</b> In Vitro Diagnostic Use	See Instruction for Use	Expiry Date	Manufacturer
Tests per Kit	Manufacturing Date	Keep Dry	Do not reuse
<b>LOT</b> Batch Number	Authorized Representative	Keep away from Sunlight	<b>REF</b> Catalog #
Store between 2~30°C			

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