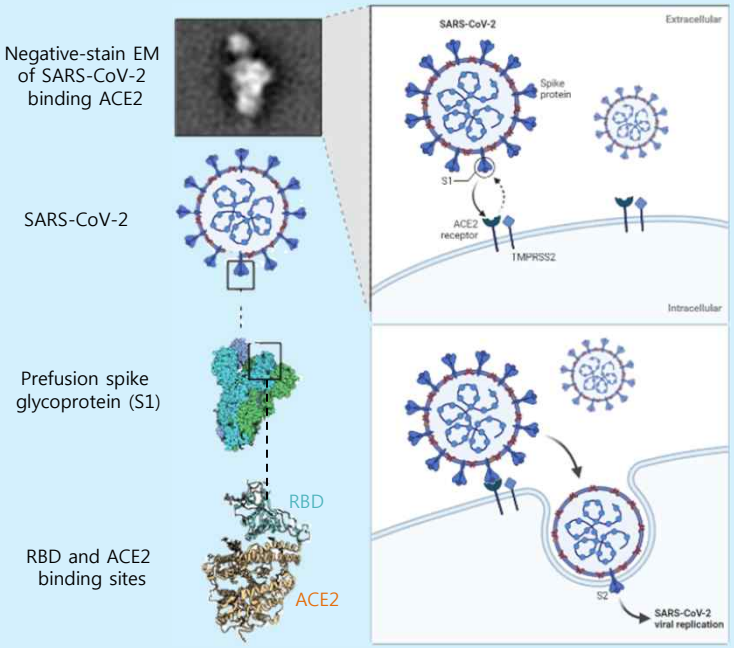
Veri-Q

SARS-CoV-2
Neutralizing Antibody
Detection ELISA Kit

Quick Protocol

Background

SARS-CoV Entry through Host ACE2

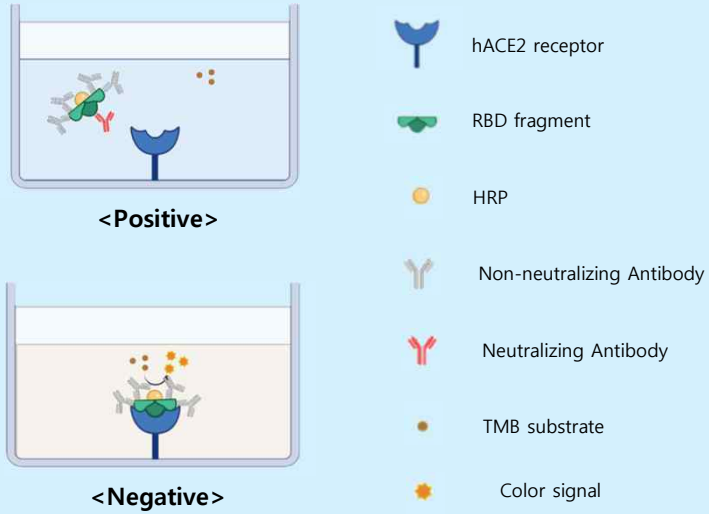


SARS-CoV-2 and SARS-CoV, which belong to the beta-CoV general of Coronaviridae, share the same receptor — ACE2 for viral entry into host cells through the S proteins on the virus surface. The S protein is composed of two subunits S1 and S2. The RBD protein of S1 protein recognizes to the cell surface receptor ACE2. It found that the RBD strongly interacted with the ACE-2 receptor, causing mainly infection of human respiratory cells. This RBD-ACE2 interaction can be neutralized (that is, blocked) by specific neutralization antibody in patient sera. Following an infection, which develop the antibodies against SARS-CoV-2. Among these binding antibodies, antibodies which can block cellular infiltration and replication of virus are called neutralization antibodies.

The Veri-Q SARS-CoV-2 Neutralizing Antibody Detection ELISA kit is a qualitative immunoassay for the detection of neutralization antibodies against SARS-CoV-2.

Assay Principle

Scheme of SARS-CoV-2 Neutralizing Ab ELISA



Competitive ELISA

This is a method of dispensing the analyte of the sample and the antibody of the solution After coating the standard antigen on the plate. In general, the obtained signal and the concentration of the target analyte are inverse proportional.

Neutralizing antibody kit

Human receptor protein (hACE2) is used as a standard antigen, and a neutralizing antibody plays the role of the sample's analyte. Kit Uses the principle that the interaction between RBD HRP and hACE2 is blocked by neutralizing antibodies.

Warning and Precaution

Warning

1) For in vitro diagnostic use.
2) This kit is intended only for the presence of total neutralizing antibodies against SARS-CoV-2, not for virus or pathogens.
3) Results confirmed by this kit cannot be used to confirm disease.
4) Strictly follow the test procedure, precautions and interpretation of results
5) Do not re-use.
6) Do not use if the product seal or its packaging is compromised.
7) Do not use after the expiration date shown on the pouch.
8) Do not mix and interchange different specimens.
9) Wear protective clothing such as laboratory coats, disposable gloves and eye protection while handling potentially infectious materials or performing the assay.
10) Wash hands thoroughly after finishing the tests.
11) Clean up spills thoroughly with appropriate disinfectants. Observe established precautions against microbiological hazards throughout testing procedures.
12) Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.

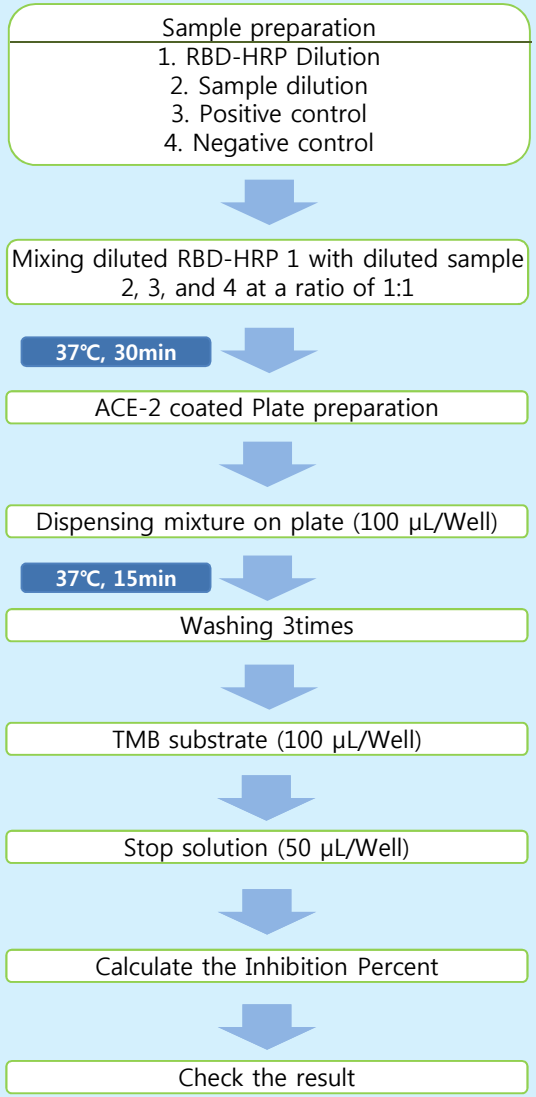
Precaution

1) Do not use after the expiration date.
2) Do not mix components from different batches or other manufacturers.
3) Prior to use, allow all reagents to come to room temperature.
4) All reagents should be mixed gently and thoroughly prior to use.
5) Incubation times or temperatures other than those stated in this insert may affect the results.
6) Store any unused antigen-coated strips in the foil Ziploc bag with desiccant to protect from moisture.
7) The light-sensitive reagents should always be stored in the light-protected bottle provided.

Storage and stability

1) If stored at 2 to 8 °C the unopened kit is stable for 6 months from the date of manufacture. And the opened kit is stable for up to 30 days (1month) form the date of opening at 2 to 8 °C.
2) This kit is stable until the expiration date printed on the package.

Procedure Summary



Product contents

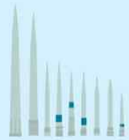
Components name	Volume	Quantity 96 tests/kit	Description
ACE2 coated plate	-	1 plate	96 well plate coated with ACE2 receptor
RBD-HRP (2000X)	30 µL	1 vial	HRP conjugated RBD containing buffer
Sample Diluent	20 mL	1 bottle	Sample dilution buffer
RBD-HRP Diluent	20 mL	1 bottle	RBD-HRP dilution buffer
Positive Control	100 µL	1 vial	Positive control
Negative Control	100 µL	1 vial	Negative control
10X Wash Buffer	20 mL	1 bottle	Washing buffer
TMB Solution	15 mL	1 bottle	Activates HRP conjugated RBD
Stop Solution	10 mL	1 bottle	Reaction stopping buffer
Plate Sealer	-	3 ea	Prevents 96 plate from contamination
Quick Guide		1 ea	

Material Required but Not Provided

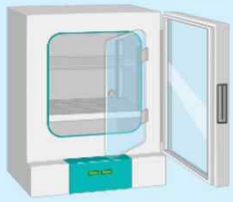
1.5mL, 15 mL tube

Micro pipette
(10 µL, 200 µL, 1000 µL)

Multichannel pipette



Pipette tips
(10 µL, 200 µL, 1000 µL)

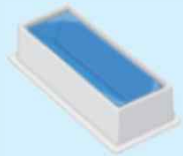


Incubator

Timer and paper towel

Deionized
/ distilled Water

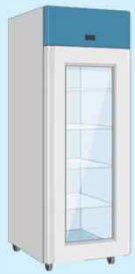
Spectrophotometric microplate reader



Disposable reagent reservoir

Powder-free gloves

Microplate washer
(Automated recommended)



Lab refrigerator

Vortex

Specimen Collection

The Veri-Q SARS-CoV-2 Neutralizing Antibody Detection ELISA kit is designed for use serum or plasma

[Serum]

Collect the blood into the commercially available without anticoagulant tube and allow it to clot

Centrifuge blood to get serum specimen of supernatant.

The serum may be stored at 2°C to 8°C for up to three days if the tests cannot be performed immediately. Allow sample to attain room temperature (without heating) prior to use.

For prolonged storage, it should be at below -20°C

[Plasma]

Collect the venous whole blood into the commercially available anti-coagulant tube such as heparin, EDTA, Sodium citrate by venipuncture.

Centrifuge blood to get plasma specimen of supernatant.

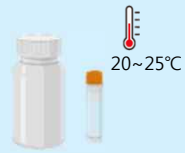
The serum may be stored at 2°C to 8°C for up to three days if the tests cannot be performed immediately. Allow sample to attain room temperature (without heating) prior to use.

For prolonged storage, it should be at below -20°C

* Note: Separate serum or plasma from whole blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens

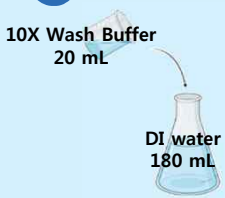
Reagent and Sample Preparation

1



Let the reagents at room temperature (20° to 25°C) about 30 minutes.

2



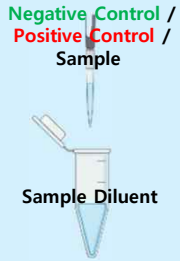
Dilute 20 mL of 10X Wash Buffer to a final volume of 200mL of distilled/deionized water. (1:10 dilution)

3

Dilute RBD-HRP with 1:2,000 dilution ratio with RBD-HRP Diluent

[Example: 5 µL RBD-HRP (2000X) in 10 mL RBD-HRP Diluent]

4



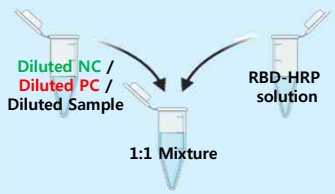
Dilute Sample, Positive and Negative Controls with a 1:10 dilution ratio with the Sample Diluent.

[Example: 15 µL Sample, Negative and Positive Control in 135 µL sample diluent]

* Positive, negative control should be assay in duplicate

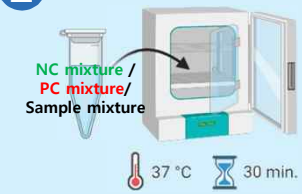
Step 2. Assay Procedure

1



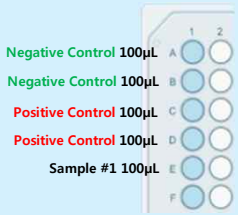
Mix the diluted control and diluted sample with diluted RBD-HRP solution with a volume ratio of 1:1.
[Example: mix 150 µL each control diluted control and diluted sample with 150 µL RBD-HRP solution.]

2



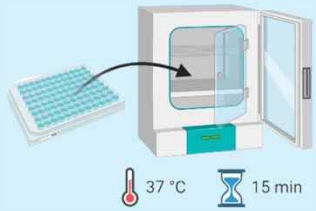
Incubate the mixtures at 37±1 ºC for 30 minutes.

3



Dispense 100 µL of mixtures corresponding well.

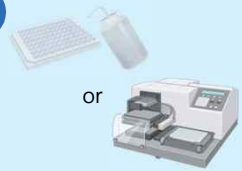
4



Seal the plate and incubate at 37±1 ºC for 15 minutes.

Step 2. Assay Procedure

5

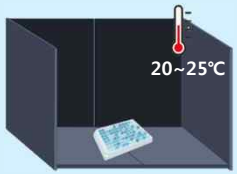


Aspirate each well and wash with 300 µL of 1X Wash Buffer 3 times using multichannel pipette or microplate washer.

6

Add 100µL of TMB solution into each well.

7



Seal the plate and incubate at room temperature for 15 minutes. Keep the plate away from light.

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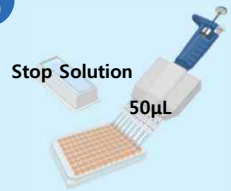
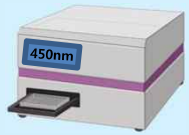


Add 50 µL of Stop Solution into each of the wells.

9



Read the plate at 450nm within 10 minutes with a microplate reader.

Step 3. Data Analysis

Validation criteria

Positive and Negative controls must include to assure the validity of results. Following the table, the average optical density (O.D.450nm) of controls must fall within the values. If these requirements are not met, the test should be repeated.

Control	O.D Requirement
Positive Control	absorbance value < 0.1
Negative Control	absorbance value > 1.0

Calculation of results

1. Calculate the mean absorbance of the duplicates of the Positive, Negative control.
2. Calculate the Inhibition Percent.

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

3. Interpretation of results

Inhibition Percent	Result	Interpretation
≥ 30% Inhibition	Positive	Neutralization antibodies for SARS -CoV-2 are detected
< 30% Inhibition	Negative	Neutralization antibodies for SARS -CoV-2 are not detected

MiCo BioMed Co., Ltd.
3rd and 4th Floor, 54 Changeop-ro, Sujeong-gu, Seongnam-si, Gyeonggi-do, Republic of Korea. 13449
E-mail. salesbio@micobiomed.com
TEL : +82-70-5227-6000~600l FAX : +82-70-5227-6001~2