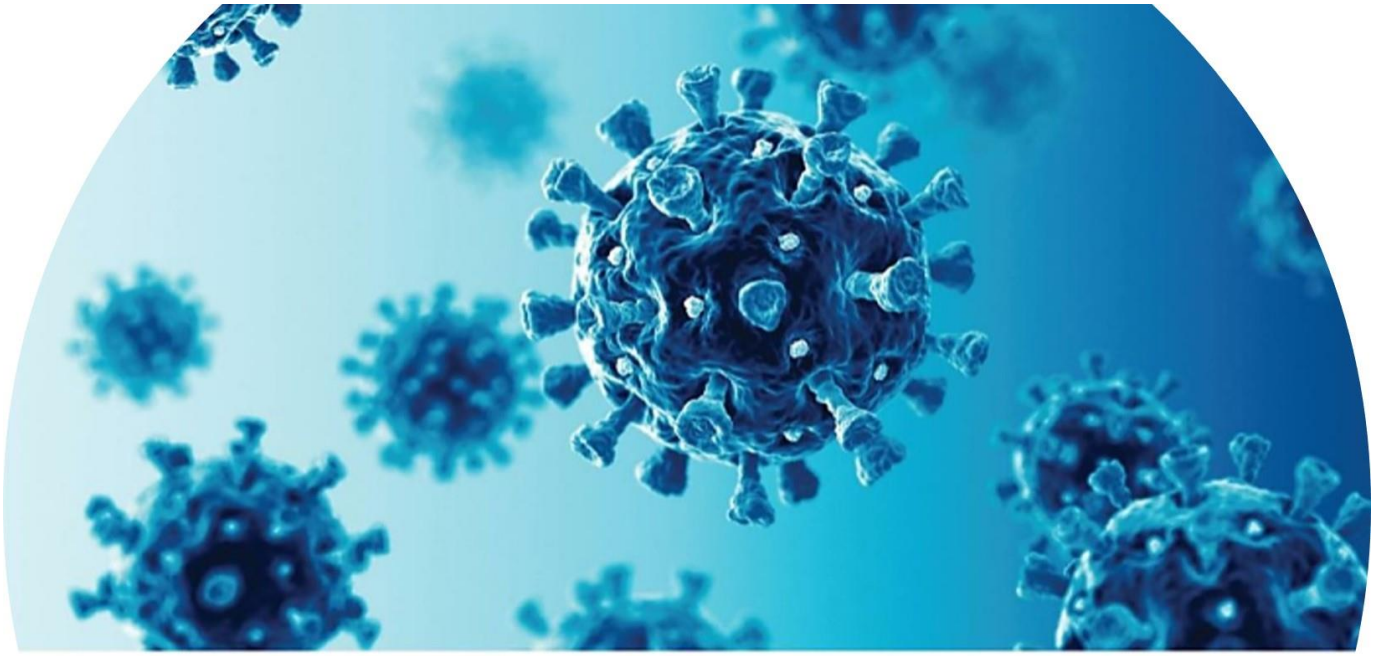
MANUAL



Malang Malang™ SARS-CoV-2
Neutralization Antibody
ELISA KIT

Cat. No. HC-NT001

For research use only. Not for diagnostic use

Version: RUU.00

Update: 2020.12.21

HANCOM HEALTHCARE

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1. Intended USE

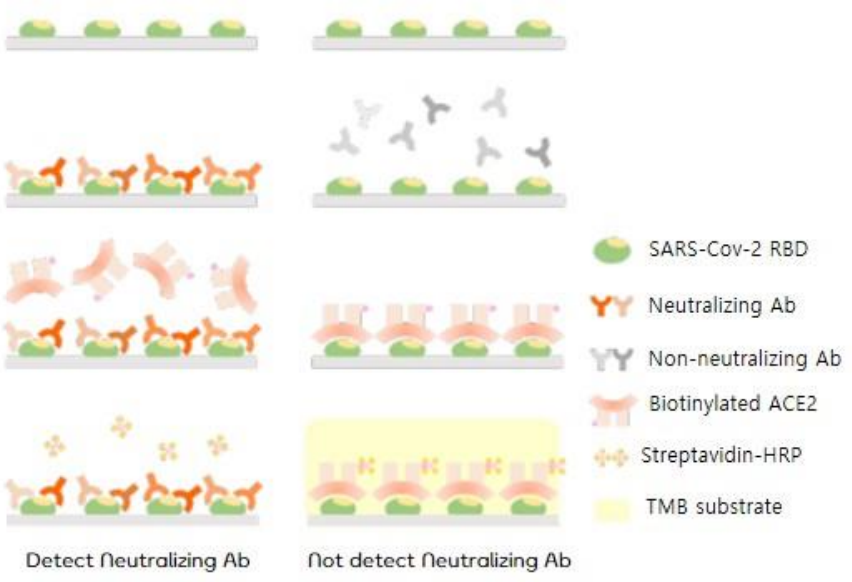
Malnag Malang™ SARS-CoV-2 Neutralization Antibody ELISA KIT is developed for detecting neutralizing antibodies against SARS-CoV-2 present in the serum and plasma. It is a safer, Faster and Easier surrogate viral Neutralization Test (sVNT) that can semi-quantify the levels of neutralizing antibodies in patients infected with SARS-CoV-2 previously or in the vaccinated group with newly developed for SARS-CoV-2. **Malnag Malang™ SARS-CoV-2 Neutralizing Antibody Assay Kit** should be used for research only.

2. Introduction

Coronaviruses (CoVs) belong to the *Coronaviridae* family, and enveloped single-stranded positive-sense RNA viruses. It mainly causes respiratory or gastrointestinal tract diseases in diverse mammals and birds (1). Recently, a worldwide COVID-19 (coronavirus disease 2019) outbreak is occurred by SARS-CoV-2 in the genus β-coronavirus (2). The structural protein of SARS-CoV-2 consists of Envelope (E), Membrane (M), Nucleocapsid (N) and Spike (S). The Spike protein is a transmembrane protein, contains two subunits (S1 and S2). Especially, a Receptor Binding Domain (RBD) located in the S1 subunit recognizes and binds to host epithelial cell surface receptor (Angiotensin-Converting Enzyme 2, ACE2), followed to facilitate the infection to human respiratory cells (3). A lot of serology studies show that specific antibody against RBD of Spike protein, called as 'Neutralizing Antibody (NA)' can block virus infection into cells and potentially protect a human from SARS-CoV-2 (4). Recently, a research group report that the interaction between RBD of SARS-CoV-2 and ACE2 receptor can be mimicked in vitro and then, the surrogate virus neutralization test (sVNT) is developed using this biochemical simulation (5). As sVNT is safer, easier, and faster than conventional virus neutralization test (cVNT). this new assay has earned even the first FDA EUA.

3. Test Principle

Malnag Malang™ SARS-CoV-2 Neutralizing Antibody Assay Kit is colorimetric kit, which is like standard ELISA using competition reaction of ACE2 receptor protein and Neutralizing Ab in the ELISA plate pre-coated with RBD protein. Principle of this assay is summarized in the schematic figure below. (For detailed experimental steps, please refer to '10. ASSAY PROCEDURE')



Serum or Plasma	Neutralizing Ab (o)	Neutralizing Ab (X)
Block of the Binding RBD-ACE2	O. K	Not
TMB substrate	No reaction	Turn the Blue

4. Component

Component	Quantity	Storage
RBD pre-coated Microplate	1 plate	4℃
Calibrator, Ready-to-use	250ul x 2vials	4℃
Positive Control, Ready-to-use	250ul x 2vials	4℃
Negative Control, Ready-to-use	250ul x 2vials	4℃
100x Detector (ACE2-biotin)	110ul x 1vial	4℃
100x SA-HRP	110ul x 1vial	4℃
10x Assay buffer	5ml x 1bottle	4℃
20x Wash buffer	30ml x 1bottle	4℃
TMB Solution	12ml x 1bottle	4℃
Stop Solution	6ml x 1bottle	4℃
Plate Covers	2ea	4℃

Unopened kits are stable for at least 6 months from the date of manufacture when stored at 2℃ to 8℃, and opened kits are stable for up to 1 month from the date of opening at 2℃ to 8℃.

5. Materials Required but Not Supplied

- Microplate reader at 450nm
- Deionized or distilled water
- Plastic container to store Wash Solution
- 96well U type plate to dilute samples
- Calibrated Precision Single and Multichannel pipette
- Disposable Pipette tips
- Disposable reagent reservoir
- Plate washer: Automated or Manual
- Graduated cylinders
- Paper towels
- Centrifuge

6. Precaution

1) All specimens originated from human should be considered as potentially infectious. Strict compliance with GLP (Good Laboratory Practice) regulations can help keep the personal safety.

2) Do not mix leftover reagents and component from other manufacturers.

3) TMB solution may cause skin, eye, and respiratory irritation.

4) Keep TMB solution protected from light.

5) The Stop Solution include sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

6) Bacterial or fungal contamination of specimens or reagents, or cross-contamination between reagents may cause erroneous results.

7) Some components contain a preservative which may cause an allergic skin reaction.

8) When reading the results, make sure that the bottom of the plate is dry and that there are no air bubbles inside the well.

7. Sample Collection & Storage

• Serum

- Use a serum separator tube (SST). Let samples clot at room temperature for 30 minutes, then centrifuge for 10 minutes at 1000xg. Assay with freshly prepared serum or store serum in aliquot at ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.

• Plasma

- Collect plasma using heparin, citrate or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay with freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/ thaw cycles.

Note:

- *Vortex serum or plasma samples at room temperature to ensure homogeneity. Then centrifuge samples at 10,000 rpm for 5 minutes prior to assay to remove particulates. Please do not omit this centrifugation step if samples are cloudy and contain particles.*
- *Heat inactivation at 56°C for 30 minutes is optional, but serum / plasma used should all treated the same way (either heat inactivated or not).*
- *Severe hemolytic samples should not be used.*
- *Sample safety: All sample should be considered as potentially infectious. Strict compliance with GLP (Good Laboratory Practice) regulations*

8. Reagent Preparation & Storage

1) Bring all reagents to room temperature before use. Leave all components to sit for a minimum of 15 minutes with gentle agitation. Working dilutions should be prepared and used immediately. Prepare enough for several additional wells for pipetting losses.

2) 1x Assay buffer at 2 to 8℃.

3) 1x Wash buffer

- Dilute 30ml of 20x Wash Concentrate with 570ml of distilled or deionized water. Store diluted Wash buffer at 2 to 8℃.

4) 1x Detector

- Dilute 100x Detector 1:99 with 1x Assay buffer (100-fold dilution). For example, dilute 100ul of 100x Detector with 9.9ml of 1xAssay buffer for one 96well plate testing.

5) 1x SA-HRP

- Dilute 100x SA-HRP 1:99 with 1x Assay buffer (100-fold dilution). For example, dilute 100ul of 100x SA-HRP with 9.9ml of 1xAssay buffer for one 96well plate testing.

9. Sample Preparation

Dilute test samples 1:9 with 1x Assay buffer (10-fold dilution). For example, Dilute 12ul of sample with 108ul of 1x Assay buffer and mix well.

10. Assay Procedure

1) Add 100ul of the calibrator, positive control, negative control, and diluted samples to the wells to be measured.
2) Seal the plate with plate cover and Incubate at room temperature for 30 minutes.
3) Wash step: Thoroughly aspirate the contents of each well. Add 300ul of 1x wash buffer to the wells to be measured. Repeat this sequence twice for a total of three washes. Invert the plate and tap it on paper towel to remove residual liquid after the last wash. Do not dry wells completely.

4) Add 100ul of diluted 1x detector to each well and Incubate the plate for 30 minutes at room temperature. Then, Seal the plate with plate cover.
5) Remove the plate cover and Wash step: Repeat step 3).
6) Add 100ul of diluted 1x SA-HRP to each well and Incubate the plate for 15 minutes at room temperature. Then, Seal the plate with plate cover.
7) Remove the plate cover and Wash step: Repeat step 3).
8) Add 100ul of TMB solution to each well and Incubate the plate in the dark at room temperature for 15 minutes.
9) Add 50ul stop solution to each well to quench the reaction.
10) Read the absorbance (OD) of each well at 450 nm immediately using microplate reader.

11. Interpretation of Result

For interpretation of inhibition rate, the positive cutoff and negative cutoff can be used in COVID-19 neutralizing antibody detection. Results can be evaluated by calculating a ratio of the OD value of the control or patient samples over the OD value of the calibrator. The operator can determine the result of the sample by comparing the ratio as follows:

$$\text{Ratio} = \frac{\text{Optical Density value of Control or Patient samples}}{\text{Optical Density value of Calibrator}}$$

Ratio	Result	Interpretation
< 0.8	Positive	Neutralizing antibodies to SARS-CoV-2 are detected
≥ 0.8	Negative	Neutralizing antibodies to SARS-CoV-2 are not detected

12. Quality Control

To assure the validity of the results, each assay should include the value of both positive and negative controls. The ratio of each control must be within the ranges below table data. If ratio of controls does not meet the requirements, the test is invalid and must be repeated.

Items	Ratio	Result of Control
Quality Control	< 0.8	Positive Control
	≥ 0.8	Negative Control

13. Clinical Performance

To validate the clinical performance of **Malnag Malang™ SARS-CoV-2 Neutralization Antibody ELISA KIT**, the comparator cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (1st FDA EUA) was used. The clinical agreement study evaluated previously frozen 64 samples, 24 SARS-CoV-2-positive serum samples and 40 SARS-CoV-2-negative serum samples confirmed through a nucleic acid amplification test (NAAT).

Clinical Agreement using cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit

		cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit	
		Positive	Negative
Malnag Malang™ SARS-CoV-2 Neutralization Antibody ELISA KIT	Positive	24	0
	Negative	0	40
	Positive Percent Agreement	99.9% (95% CI 86.3% - 99.9%)	
	Negative Percent Agreement		99.9% (95% CI 91.2% - 99.9%)
	Overall Percent Agreement	100 x [(24+40)/64] = 100%	

14.General References

(1) Coronaviruses: an overview of their replication and pathogenesis. Anthony R Fehr, et al.; Methods Mol Biol. ;1282:1-23 (2015).

(2) A pneumonia outbreak associated with a new coronavirus of probable bat origin: P. Zhou, et al.; Nature 579, 270 (2020)

(3) SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor: M. Hoffmann, et al.; Cell 181, 271 (2020)

(4) The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients: L. Premkumar, et al.; Sci. Immunol. 48, eabc8413 (2020)

(5) A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction: Chee Wah Tan et al.; Nat Biotechnol. 9,1073-1078 (2020)