Anti-SARS-CoV-2 Neutralizing Antibody (NtAb) ELISA Kit

Catalog No.: BEK1265

Size: 96T

Storage and Expiration: Store at 2-8℃ for 6 months.

1. **Store Positive Control, Negative Control and HRP conjugated S-RBD antibody at -20℃.**
2. **It is recommended to use the kit within 1 month once open.**

Application: For qualitative detection of any antibodies that neutralize the interaction of SARS-CoV-2 Spike RBD-ACE2 in serum, plasma.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, or 2019-nCoV) is an enveloped non-segmented positive-sense RNA virus. It is the cause of coronavirus disease 2019 (COVID-19), which is contagious in humans. SARS-CoV-2 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 SARS-CoV-2 S protein strongly interacts with the human ACE2 receptor leading to endocytosis into the host cells of the deep lung and viral replication. Infection with the SARS-CoV-2 initiates an immune response, which includes the production of antibodies in the blood. The secreted antibodies provide protection against future infections from viruses, because they remain in the circulatory system for months to years after infection and will bind quickly and strongly to the pathogen to block cellular infiltration and replication. These antibodies are named neutralizing antibodies.

This kit can detect circulating neutralizing antibodies against SARS-CoV-2 that block the interaction between the receptor binding domain of the viral spike glycoprotein (RBD) with the ACE2 cell surface receptor. The detection is not limited by both species and antibody isotypes.

Principle of the Assay

The kit is based on the competitive ELISA, it is a blocking ELISA detection tool, which mimics the virus neutralization process. The kit contains two key components: HRP conjugated recombinant SARS-CoV-2 Spike RBD fragment protein (HRP-SRBD) and the human ACE2 receptor protein (hACE2). The protein-protein interactions between HRP-SRBD and hACE2 can be blocked by neutralizing antibodies against SARS-CoV-2 Spike RBD. Incubate the samples and controls with HRP-SRBD to make the combination of the neutralization antibodies with HRP-SRBD. Then, add the mixture to the hACE2 pre-coated plate. The unbound HRP-SRBD as well as any HRP-SRBD bound to non-neutralizing antibody will be captured on the plate, while the neutralizing antibodies-HRP-SRBD complexes remain in the supernatant and were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is inverse to the Anti-SARS-CoV-2 Neutralizing Antibody amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of Anti-SARS-CoV-2 Neutralizing Antibody can be calculated.

Chongqing Biospes Co., Ltd Tel: +86-23-67567091 Fax: +86-23-67745923
7F, Bldg B, High-tech Venture Park, # 107 Erlang Chuangye Rd, Jiulongpo District, Chongqing, 400039, China
www.biospes.com

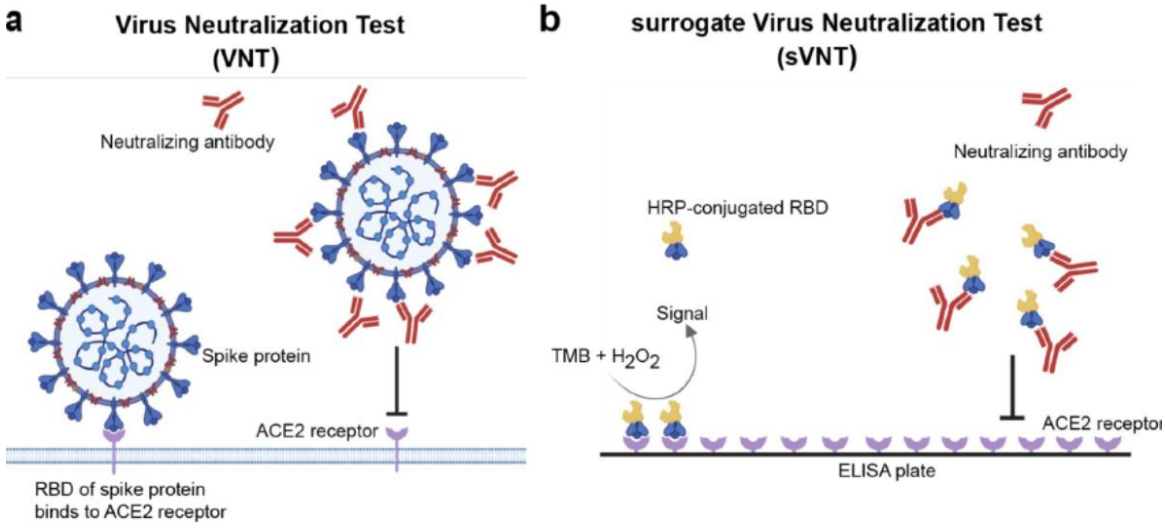

Figure 1: From Chee Wah Tan, Wan Ni Chia, Mark I-C Chen, Zhiliang Hu, Barnaby E. Young, Yee-Joo Tan, Yongxiang Yi, David C. Lye, Danielle E. Anderson, Lin-Fa Wang. A SARS-CoV-2 surrogate virus neutralization test (sVNT) based on antibody-mediated blockage of ACE2-spike (RBD) protein-protein interaction. DOI: https://doi.org/10.21203/rs.3.rs-24574/v1

Kit components

1. One 96-well pre-coated plate
2. One 96-well plate for dilution
3. Positive control: 15 μl (100 ×)
4. Negative control: 150 μl (10 ×)
5. HRP conjugated SARS-CoV-2 Spike RBD protein (HRP-SRBD): 20 μl (1000 ×)
6. Diluent buffer: 30 ml + 10 ml
7. Wash buffer: 30 ml (10×)
8. TMB substrate A: 10 ml
9. TMB substrate B: 10 ml
10. Stop Solution: 10 ml
11. Plate sealer: 4 sheets
12. Hermetic bag: 1 pcs

Material Required But Not Provided

1. Microplate reader (wavelength: 450 nm)
2. 37℃ incubator
3. Automated microplate washer
4. Deionized or distilled water
5. Graduated cylinder to prepare wash solution
6. Plastic/glass container to prepare wash solution
7. Precision pipettes to deliver 1 μl, 10 μl, 100 μl, 200 μl and 1000 μl content
8. 10 μl, 100 μl, 200 μl and 1000 μl pipette tips
9. Multichannel pipettor
10. Disposable reagent reservoirs

Chongqing Biospes Co., Ltd Tel: +86-23-67567091 Fax: +86-23-67745923
7F, Bldg B, High-tech Venture Park, # 107 Erlang Chuangye Rd, Jiulongpo District, Chongqing, 400039, China
www.biospes.com

Protocol

- **Preparation of sample and reagents**

1. Sample collection

Isolate the test samples soon after collecting, then, analyze immediately (within 8 hours). Or aliquot and store at -20℃ for long term. Avoid multiple freeze-thaw cycles.

✧ **Serum:** Use disposable medical blood vessels (without anticoagulant) to collect blood 2-5 ml, place at room temperature (18-25℃) for 30-60 min or 2-8℃ overnight, then, centrifugate at 3000 r.p.m. for 10 min to separate serum.

✧ **Plasma:** Use disposable medical anticoagulant tube to collect blood 2-5 ml, centrifugate at 3000 r.p.m. for 10 min to separate plasma to avoid hemolysis.

>> Sample working solution

Dilute the samples with Diluent buffer at 1:10. i.e. Add 10 µl of samples into 90 µl of Diluent buffer.

2. Positive control (Kit Component 3) working solution: Dilute the Positive control with Diluent buffer at 1:100. i.e. Add 1 µl of Positive control into 99 µl of Diluent buffer).

3. Negative control (Kit Component 4) working solution: Dilute the Negative control with Diluent buffer by volume ratio of at 1:10. i.e. Add 10 µl of Negative control into 90 µl of Diluent buffer.

4. HRP conjugated SARS-CoV-2 Spike RBD protein (Kit Component 5) working solution: Dilute the HRP conjugated SARS-CoV-2 Spike RBD protein with Diluent buffer at 1:1000. i.e. Add 10 µl of HRP conjugated SARS-CoV-2 Spike RBD protein into 9990 µl of Diluent buffer.

5. Wash Buffer (Kit Component 7) working solution: Equilibrate the Wash Buffer (10 ×) to room temperature (18-25℃) before use, and shake to dissolve salt precipitation if there is. Dilute the Wash buffer (10 ×) at 1:10 with distilled water. i.e. Add 30 ml of concentrated wash buffer into 270 ml of distilled water.

- **Assay procedure**

It is recommended to analyze Positive Control, Negative Control and samples in duplicate.

1. On the 96-well plate for dilution (Kit Component 2), mix the Positive control, Negative control and test samples working solution with the HRP-SRBD working solution respectively at 1:1. i.e. Mix the 75 µl of Positive control working solution with 75 µl of HRP-SRBD working solution (#M1), mix 75 µl of Negative control working solution with 75 µl of HRP-SRBD working solution (#M2) and mix 75 µl of test samples working solution with 75 µl of HRP-SRBD working solution (#M3).
2. Cover the plate with the provided Plate sealer (Kit Component 11) and incubate the mixtures at 37℃ for 30 min.
3. Set test sample, positive and negative controls wells on the 96-well pre-coated plate (Kit Component 1) respectively, and then, record their positions. Take the corresponding strips out from the plate holder, and store the unused strips at 2-8℃ in their pouch or the provided Hermetic bag (Kit Component 12).
4. Add 100 µl of the #M1, #M2 & #M3 into the corresponding wells of the 96-well pre-coated plate (Kit Component 1).
5. Cover the plate with the provided Plate sealer (Kit Component 11) and incubate at 37℃ for 30 min.
6. Remove the sealer, and discard the plate content, clap the plate on the absorbent filter papers or

Chongqing Biospes Co., Ltd Tel: +86-23-67567091 Fax: +86-23-67745923
7F, Bldg B, High-tech Venture Park, # 107 Erlang Chuangye Rd, Jiulongpo District, Chongqing, 400039, China
www.biospes.com

other absorbent material. **Try to avoid the cross contamination between different wells.** And wash plate using one of the following methods:

Manual Washing: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers. Fill each well completely with Wash Buffer (1x) and vortex mildly on ELISA shaker for 2 min, then aspirate contents from the plate, and clap the plate on absorbent filter papers. Repeat this procedure four more times for a **total of FIVE washes**.

Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1x). After the final wash, invert plate, and clap the plate on absorbent filter papers until no moisture remained. It is recommended that the washer be set for a soaking time of 10 seconds or shaking.

7. Mix TMB substrate A and TMB substrate B thoroughly at 1:1. Then, add 100 µl of the mixture into each well.
8. Cover the plate with the provided Plate sealer (Kit Component 11) and incubate at 37℃ for 8-10 min.

Note: If the Negative Control colour is relatively light, prolong the reaction time appropriately.

9. Add 50 µl of Stop solution (Kit Component 10) into each well and mix thoroughly to terminate the reaction.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 5 min after adding the stop solution.

- **Results analysis**

To ensure the validity of the results, both Positive and Negative Controls must be included for each assay. The net $O.D._{450}$ of each control must fall within the ranges listed in the following table. If not, the assay is invalid and should be analyzed again.

1. Net $O.D._{450}$ reference

Items	Control Result for Valid Assay	$O.D._{450}$ value
Quality Control	Negative control	> 1.2
	Positive control	< 0.1

Note: The above reference is for evaluating the performance of the kit only.

2. Interpretation of results

The cutoff value for positive and negative of SARS-CoV-2 neutralizing antibody can be explained by inhibition rate.

Inhibition = (1 -$O.D._{450}$ value of Samples / $O.D._{450}$ value of Negative Control) × 100%

Cutoff Interpretation**

Items	Cutoff	Result	Interpretation
SARS-CoV-2 neutralizing antibody test	≥ 10%	Positive	SARS-CoV-2 neutralizing antibody was detected
	< 10%	Negative	SARS-CoV-2 neutralizing antibody was not detected

****The cutoff value is based on validation of COVID-19 recovered patient (confirmed and diagnose by native hospitals) and negative / healthy people serum, so, it is for reference use only. End users can set their own cutoff value according to the serum of patients from different geographic**

locations or different races.

For evaluating the effect of the vaccine, it is recommended to compare the serum from the same person before and after vaccination to assess whether the neutralizing antibodies have been produced.

Precautions

1. Handle all biological material as potentially infectious.
2. The recombinant ACE2 protein was not pre-coated on the dilution plate. Dilution plate is used for dilution of samples, Negative and Negative control only. Disposable EP tubes can also be used to replace it.
3. Wear Laboratory clothing, disposable gloves, and masks when handling samples and reagents.
4. The pre-coated plate of this kit is disposable and cannot be used repeatedly.
5. Positive and Negative control should be included for each assay.
6. Do not reuse pipette tips and tubes to avoid cross contamination.
7. Do not contact directly with TMB substrate and stop solution, they may be irritating to the skin, eyes and respiratory tract.
8. Do not expose TMB substrate to strong light or any oxidizing agents.
9. To avoid the marginal effect of plate incubation for temperature differences (the marginal wells always get stronger reaction), it is recommended to equilibrate the reagents for use to room temperature (18-25℃) before assay. Pipette the proper volume of the using reagents, and store the rest back at 2-8℃. **Do not return the reagents into their original vials once pipette!**
10. All samples and controls should be vortexed and centrifuged prior to use.
11. All wastes should be properly decontaminated prior to disposal and the dispose should be in accordance with local, regional or national regulations.
12. Do not use the expired components and the components from different batches.
13. ONLY deionized or distilled water can be used to dilute wash buffer (10 x) during the assay.
14. The unused strips should be sealed with its foil pouch and store at 2-8℃.

Reference

1. Chee Wah Tan, Wan Ni Chia, Mark I-C Chen, Zhiliang Hu, Barnaby E. Young, Yee-Joo Tan, Yongxiang Yi, David C. Lye, Danielle E. Anderson, Lin-Fa Wang. A SARS-CoV-2 surrogate virus neutralization test (sVNT) based on antibody-mediated blockage of ACE2-spike (RBD) protein-protein interaction. DOI: https://doi.org/10.21203/rs.3.rs-24574/v1