Human SARS-CoV-2 Neutralizing Antibody ELISA Kit

Catalog No: abx365044

Size: 96T

Range: Qualitative

Sensitivity: Qualitative

Storage: Store at 2-8°C for 6 months.

Application: The qualitative detection of Human SARS-CoV-2 Neutralizing Antibody in Human serum, plasma and other biological fluids.

Principle of the Assay: This kit is based on competitive enzyme-linked immuno-sorbent assay technology. Human ACE2 is pre-coated onto a 96-well plate. Controls, test samples, and HRP-conjugated detection reagent are added to the wells and incubated. The ACE2 in the wells competes with neutralizing antibody in the sample for binding sites on the detection reagent. Unbound conjugates are removed using wash buffer at each stage. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient SARS-CoV-2 Neutralizing Antibody will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow colour is proportional to SARS-CoV-2 Neutralizing Antibody amount bound on the plate. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the presence of SARS-CoV-2 Neutralizing Antibody can be determined.

Kit Components

- Pre-coated 96-Well Microplate: 12 x 8
- Plate Sealer: 3
- Hermetic Bag: 1
- Positive Control: 2
- Negative Control: 2
- Detection Reagent: (100X) 120 µl
- Sample/Standard Diluent Buffer: 20 ml
- Diluent Buffer: 14 ml
- Wash Buffer: (25X) 30 ml
- Stop solution: 10 ml

Materials Required But Not Provided

- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Microplate reader (wavelength: 450 nm)
- ELISA Shaker

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Protocol

A. Sample Preparation

Analyse immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 1000 × g for 20 mins. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 mins at 1000 x g, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples.
- **Other Biological Fluids:** Centrifuge at approximately 1000 × g for 20 mins to remove precipitate. Analyse immediately or aliquot and store at -20°C or -80°C.

Notes:

- **Analyse samples at 1/10 dilution (i.e. add 10 µl of sample to 90 µl of Sample/Standard Diluent Buffer).**
- Store frozen samples undiluted. Once ready to analyse, thaw samples and dilute.
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- NaN_3 cannot be used as a test sample preservative, since it inhibits HRP.
- If a sample is not indicated in the manuals applications, a preliminary experiment to determine the suitability of the kit will be required.

B. Reagent Preparation

Wash Buffer: Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water). If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

Detection Reagent Working Solution Preparation: Prepare no more than 1 day before the experiment.

1. Calculate the total volume of working solution required.
2. Briefly centrifuge the detection reagent, then dilute it 100-fold with Diluent Buffer, and mix thoroughly. Pipette with a slow, smooth action to reduce volume errors.

Control Solution Preparation: Prepare no more than 1 day before the experiment.

1. Briefly centrifuge the Positive Control, and then carefully dissolve in 0.3 ml of Sample/Standard Diluent Buffer.
2. Briefly centrifuge the Negative Control, and then carefully dissolve in 0.5 ml of Sample/Standard Diluent Buffer.

C. Assay Protocol

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate.

1. Set 2 positive and 2 negative control, test sample and control (zero) wells on the pre-coated plate respectively, and record their positions. *Add the solution to the bottom of each well without touching the side walls. Pipette the standards and samples up and down to mix before adding to the wells. Avoid foaming or bubbles.*
2. Aliquot 50 µl of negative and 50 µl positive control into the set wells. Add 50 µl of Sample/Standard Diluent Buffer to blank wells.
3. Aliquot 50 µl of appropriately diluted sample into the test sample wells. Gently tap the plate to mix, or use a microplate shaker.
4. Immediately add 50 µl of Detection Reagent working solution into each well. Cover the plate with a plate sealer and incubate for 1 hr at 37°C.
5. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (350 µl) using a multi-channel Pipette or autowasher (1-2 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.*

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6. Aliquot 90 µl of TMB Substrate into each well. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 10-20 minutes. The incubation time is for reference only, the optimal time should be determined by end user. Avoid exposure to light.
7. Aliquot 50 µl of Stop Solution into each well. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
8. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the OD at 450 nm immediately.

Data Analysis:

- Mean OD of the Positive Control should be < 0.4.
- Mean OD of the Negative Control should be > 1.2.
- Inhibition value = 1 - (OD of Sample / OD of Negative Control)

If the Postive Control value is < 0.4, and the Negative Control value is > 1.2, the test is valid, otherwise, the test is invalid.

If Inhibition < 0.2 the test samples are considered negative.

If Inhibition ≥ 0.2, the test samples are considered positive.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimised. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.
- Please note that this kit is optimised for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

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