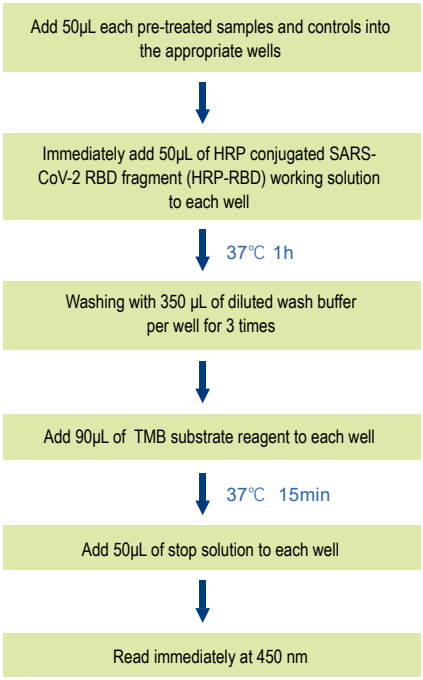
Procedure flow chart



Interpretation

The OD of the negative control is used to calculate the inhibition, and the OD of positive control is only used to evaluate the validity of the results. The inhibition of each sample can be calculated with the formulation as follows:

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

Inhibition ⩾ 20%: Positive, Neutralization antibodies for SARS-CoV-2 are detected.

Inhibition ＜ 20%: Negative, Neutralization antibodies for SARS-CoV-2 are not detected.

Limitations

1. This kit is intended for the semi quantitative detection of neutralization antibodies against SARS-CoV-2 in human serum or plasma.
2. For medical professional use only! The results should not be used as the sole basis, and should be combined with other test methods, such as molecular diagnostic and pseudovirus neutralization antibodies assay.
3. Negative results do not rule out SARS-CoV-2 infection, particularly those who have been in contact with the virus recently. Positive results may be due to current or past infection with non-SARS-CoV-2 corona virus strains. Results from this kit should not be used to diagnose or to exclude acute SARS-CoV-2 infection or to inform infection status.
4. For the suspicious samples near borderline. It is recommended to re-determine and supervise dynamically.
5. Due to methodological or immuno-specific reasons, the same sample may yield different results by using reagents from different manufacturers. Therefore, the test results of different kits should not be directly compared with each other to avoid erroneous medical interpretations. It is recommended that the laboratory shall indicate the source of the reagents used in the test report. And in continuous monitoring, additional continuity testing should be performed and parallel comparison with the original reagent results to re-determine the baseline value if the reagent type is changed.
6. Results from immunosuppressed patients should be interpreted with caution.
7. Avoid using cross-contamination, microbial contamination, severe hemolysis, or turbid samples.

Analytical performance

1. Repeatability: CV ⩽ 15%
2. Analysis specificity: There is no cross-reaction with antibody/antigen positive sera samples from patients with other human coronaviruses (HCoV-HKU1, HCoV-OC43, HCoV-NL63, HCoV-229E), or non-coronaviruses, including influenza A virus (H1N1, H3N2, H5N1, H7N9), influenza B virus (yamagata lineages, victoria lineages), respiratory syncytial virus, rhinovirus, adenovirus, enterovirus, epstein-barr virus, measles virus, human cytomegalovirus, rotavirus, norovirus, mumps virus, herpes zoster virus, or mycoplasma pneumoniae.
3. When determing the cut-off value of this kit, 500 serum samples of healthy people have been measured, take the 95% upper limit as cut-off value.

   The cut-off value : ＜ 20%

Cautions

1. For medical professional use only !
2. Read the instruction manual carefully before operation, and perform the test operation strictly following the instruction.
3. Do not eat, drink or smoke in the area where samples or kits are handled. Avoid testing in harsh environments (such as environments containing sodium hypochlorite, acid-base or acetaldehyde, and other high concentration corrosive gases and dust). Disinfection should be performed after the test.
4. Wear lab coats, eye protection and disposable gloves while handling the kit reagents and wash hands thoroughly.
5. Human source material used to prepare the controls included in this kit should be handled as potentially infectious material. Use universal precautions when handling.
6. The kit should not be used beyond the expiration date on the kit label.
7. Do not mix components from different batches. Do not mix with components from other manufacturers.
8. Do not reuse the used kit.
9. The remaining strips should be sealed in an foil pouch to prevent moisture after the microplate package is opened.
10. Change pipette tips in between adding of each control and sample. Also, use separate reservoirs for each reagent.
11. Wash the wells gently when adding wash buffer to avoid the contamination between adjacent wells.
12. Residual liquid (>10 μL) in the reagent wells after washing can interfere with the substrate and lead to false low OD readings.
13. Test incubator must be set to 37± 1℃
14. Follow the Instructions of the microplate reader for set-up and preheat it for 15 min before OD measurement.
15. Handle all samples cautiously as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of samples.
16. The used reagents, samples and potentially contaminated should be discarded according to the local regulation.

BASIC INFORMATION

GLOSSARY OF SYMBOLS

Symbol	Meaning	Symbol	Meaning
IVD	In vitro diagnostic medical device	2℃ 8℃	Temperature limitation
	Manufacturer		Authorized representative in the European Community
	Date of Manufacture		Use by date
	Do not reuse	CE	Consult instructions for use
LOT	Batch code		Meet the requirements of EC Directive 98/79/EC
Σ	Tests per kit		Do not use if package damaged

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Version: 1.0 Date Adopted: 2020-12-01

Anti-SARS-CoV-2 Neutralization Antibody Test Kit

(Enzyme-linked immunosorbent assay)

Package: 96 T/ 192 T /480 T /960 T

Catalogue No: CP04008-96 T / CP04008-192 T / CP04008-480 T / CP04008-960 T

Intended use

The Anti-SARS-CoV-2 Neutralization Antibody test Kit is an enzyme-linked immunosorbent assay intended for the semi quantitative detection of Neutralization antibodies against SARS-CoV-2 in human serum or plasma.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously designated 2019-nCoV) is the pathogen of coronavirus disease 2019 (COVID-19), which is a positive-sense single-stranded RNA virus that belongs to the family of coronaviruses. The most common symptoms of COVID-19 are fever, coughing, and breathing difficulties. The incubation period of COVID-19 commonly ranges from 2 to 14 days, with an average of 5 days, although there also have few cases with 24 days incubation periods. Normally, the time from infection onset to onset of symptoms is one week. Coronaviruses encode four major structural proteins, spike (S), membrane (M), envelop (E), and nucleocapsid(N), notably, S protein contains a receptor-binding domain (RBD) which is one of the vital immunodominant epitopes and has a superior capacity to induce Neutralization antibodies. It is proved that RBD of SARS-CoV-2 is responsible for recognizing and interacting with the cell surface receptor, angiotensin-converting enzyme-2 (ACE2). In the respiratory tract, ACE2 is widely expressed on the cell surface of alveoli, trachea, bronchi, macrophages, etc. Following the binding of the RBD to the receptor ACE2, SARS-CoV-2 enters target cells, where the fusion of the virus envelops the endosome membranes and leads to the release of the viral nucleocapsid into the cytosol of the infected cell.

Principle

It is a semi quantitative competition ELISA kit to detect the Anti-SARS-CoV-2 Neutralization Antibody in human serum or plasma.

The micro test plate provided in this kit is pre-coated with recombinant human ACE2. During the reaction, the SARS-CoV-2 neutralization antibody in the sample diluent pretreated samples or controls competes with a fixed amount of human ACE2 on the solid phase supporter for sites on the Horseradish peroxidase (HRP) conjugated recombinant SARS-CoV-2 RBD fragment (HRP-RBD). After 37℃ incubation, the unbound HRP-RBD as well as any HRP-RBD bound to non-neutralization antibody will be captured on the plate and eventually form the ACE2-RBD-HRP complex, while the circulating neutralization antibodies HRP-RBD complexes remain in the supernatant and are removed during washing. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. Compared with the inhibition ratio to judge whether SARS-CoV-2 Neutralization Antibody exists in the tested samples or not.

Components

Components	96T	192T	480T	960T
Micro test plate (Dismountable)	8 wells×12 strips	2 plates, 96T	5 plates, 96T	10 plates, 96T
Positive control	1 vial	2 vials	5 vials	10 vials
Negative control	1 vial	2 vials	5 vials	10 vials
Sample diluent	1 vial, 20 mL	2 vials, 20 mL	5 vials, 20 mL	10 vials, 20 mL
Concentrated HRP conjugated RBD(HRP-RBD, 100×)	1 vial, 120 μL	2 vials, 120μL	5 vials, 120 μL	10 vials, 120 μL
HRP conjugate diluent	1 vial, 10 mL	2 vials, 10 mL	5 vials, 10 mL	10 vials, 10 mL
Concentrated wash buffer(25×)	1 vial, 30 mL	2 vials, 30 mL	5 vials, 30 mL	10 vials, 30 mL
TMB substrate solution	1 vial, 10 mL	2 vials, 10 mL	5 vials, 10 mL	10 vials, 10 mL
Stop solution	1 vial, 10 mL	2 vials, 10 mL	5 vials, 10 mL	10 vials, 10 mL
Plate sealer	3 pieces	6 pieces	15 pieces	30 pieces
Product description	1 copy	1 copy	1 copy	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Other supplies required

- Microplate reader with 450 nm wavelength filter
- High-precision transfer pettor, EP tubes and disposable pipette tips
- Incubator capable of maintaining 37℃
- Deionized or distilled water
- Absorbent paper
- Loading slot for wash buffer
- Automatic microplate washer (recommended, the washing step can also be carried out manually)
- Timer
- Paper towel
- Disinfecting agent

Storage and stability

1. The test kit has to be stored at 2-8℃ , do not freeze and avoid exposure to direct sunlight. The unopened is stable for at least 6 months.
2. The opened kit can be stored at 2-8℃ for 2 weeks.

Preparation

1. All reagents must be brought to room temperature (18-25℃) at least 30 min before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. Wash buffer: Dilute 30mL of concentrated wash buffer with 720mL of deionized or distilled water to prepare 750mL of wash buffer.

   Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. HRP conjugated RBD: Calculate the required amount before the experiment (50μL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100×concentrated HRP-RBD to 1× working solution with HRP conjugate diluent.

   Note: The HRP-RBD working solution should be stored at 2-8℃ and used within 1 days.
4. Samples: Dilute samples with sample diluent with a volume ratio of 1:9.
5. Positive control: Dissolve positive control with 0.3mL sample diluent.
6. Negative control: Dissolve negative control with 0.5 mL sample diluent.

Sample collection and preparation

Serum: Allow samples to clot for 1 hour at room temperature or overnight at 2-8℃ before centrifugation for 20 min at 1000×g at 2-8℃ . Collect the supernatant to carry out the assay. The suspended fibrous protein may cause a false positive result if not fully precipitated. Obviously contaminated samples can't be detected.

Plasma: Collect plasma using EDTA or heparin sodium as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2- 8℃ within 30 min of collection. Collect the supernatant to carry out the assay.

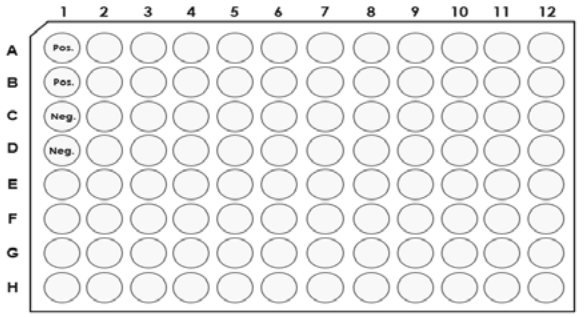
Before test, prepare the samples as the following instructions:

Sample dilution: Dilute the serum or plasma with sample diluent with a volume ratio of 1:9, mix thoroughly.

Note for samples

1. Handle all serum and plasma as if capable of transmitting infectious agents.
2. Tubes for blood collection should be disposable and be non-endotoxin. Severe hemolysis, lipoid, or turbidity samples should not be used.
3. Samples should be assayed stored at 2-8℃ within 3 days. Avoid repeated freeze-thaw cycles or overheated. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates. Frozen samples must be mixed well and brought to room temperature before testing.

Pipetting protocol



Pos.: positive control;

Neg.: negative control;

Test Procedure

1. Determine wells for positive and negative controls and samples. Add 50μL each pre-treated samples and controls into the appropriate wells (It is recommended that all samples and controls be assayed in duplicate). Immediately add 50μL of HRP conjugated SARS-CoV-2 RBD fragment (HRP-RBD) working solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 60 min at 37℃ .

   Note: solutions should be added to the bottom of the micro test plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Decant the solution from each well, add 350μL of wash buffer to each well. Soak for 30-60 seconds and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.

   Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
3. Add 90μL of substrate reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37℃ . Protect the plate from light.

   Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min. Preheat the microplate reader for about 15 min before OD measurement.
4. Add 50μL of stop solution to each well.

   Note: adding the stop solution should be done in the same order as the substrate solution.
5. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.