# Anti-SARS-CoV-2 Neutralizing Antibody ELISA Kit Instructions for Use

## [PRODUCT NAME]

Generic Name: Anti-SARS-CoV-2 Neutralizing Antibody ELISA Kit

## [SPECIFICATION]

96 tests/kit

## [INTENDED USE]

The Anti-SARS-CoV-2 Neutralizing Antibody ELISA Kit is an enzyme-linked immunosorbent assay intended for the qualitative detection of neutralizing 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 neutralizing 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 OF DETECTION]

The Anti-SARS-CoV-2 Neutralizing Antibody ELISA Kit is a blocking ELISA detection tool. The test kit contains microplate strips each with 8 wells coated with human ACE2 receptor protein (hACE2). In the first reaction step, samples are dilutedand mix with horseradish peroxidase (HRP)-labelled recombinant SARS-CoV-2 RBD protein (HRP-RBD) outside of hACE2 coated ELISA plate, neutralizing antibodies in positive samples will bind to the HRP-RBD. To detect the unbound HRP-RBD, reaction mixture is transferred into the ELISA microplate to catalyze a color reaction. The interaction between HRP-RBD and hACE2 can be blocked by neutralizing antibodies that bound to SARS-CoV-2 RBD. The final solution can be read by a microplate reader at wavelength of 450 nm, and the absorbance of the samples is inversely related to the inhibition% of the active neutralizing antibodies, and the inhibition% can be calculated according to the OD450 value of samples and negative control.



## [MAIN COMPONENTS PROVIDE IN THIS KIT]

| Components | Color | Format |
|---|---|---|
| **1.Microplate wells coated with hACE2** Ready for use | - | 12 × 8, 96 well |
| **2.96-well dilution plate** Ready for use | - | 96 well |
| **3.HRP conjugated RBD, HRP-RBD** 100 × concentrate | colorless | 100 µL |
| **4.HRP dilution buffer** Ready for use | light blue | 10 mL |
| **5.Sample dilution buffer** Ready for use | light yellow | 10 mL |
| **6.Wash buffer** 80 × concentrate | colorless | 30 mL |
| **7.Positive control** (Neutralizing antibody, human) Ready for use | colorless | 50 µL |
| **8.Negative control** Ready for use | colorless | 50 µL |
| **9.TMB Substrate solution** Ready for use | colorless | 10 mL |
| **10.Stop Solution** Ready for use | colorless | 6 mL |
| **11.Protective foil** | - | 3 pieces |
| **12.Instructions for use** | - | 1 booklet |

Note: The components in different batches of kits are not interchangeable. The format of each reagent is the minimum volume.



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Additional materials and equipment (not supplied in the test kit)

- Automated ELISA Workstation (optional)
- Microplate reader: wavelength of 450 nm
- Automatic microplate washer: recommended, the washing step can also be carried out manually.
- Microplate shaker
- Calibrated pipettes
- Pipette tips
- Distilled or deionized water
- Incubator or water bath
- Timer
- Paper towel
- Disinfecting agent

## [STORAGE CONDITIONS & SHELF LIFE]

1.The test kit has to be stored at a temperature between +2°C and +8°C, do not freeze, and avoid exposure to direct sunlight. The unopened is stable for at least 10 months.
2.The opened kit can be stored at a temperature between +2°C and +8°C for 2 weeks.

## [SPECIMEN REQUIREMENTS]

**Samples:** Human serum or plasma
1.The sediment and suspended matter in samples may interference with the test results, which should be removed by centrifugation at 6000 × g, 10 min.
2.Severe hemolysis, lipoid, or turbidity samples should not be used.
3.Samples should be stored at room temperature no longer than 8 hours, if the assay will not be completed within 8 hours, the samples should be refrigerated at +2°C to +8°C, if the samples will be stored for over 48 hours, samples should be frozen at -20°C or lower. Samples should not be repeatedly frozen and thawed or overheated.
4.Frozen samples must be mixed well after thawing and brought to room temperature (+18°C to +28°C) before testing.

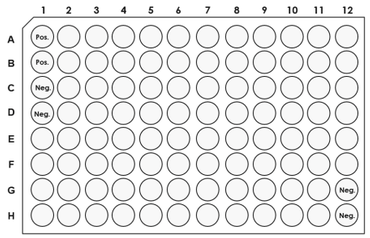




## [TEST METHODS]

**Preparation**
1.All reagents must be brought to room temperature (+18°C to +28°C) at least 30 minutes before use. Keep all reagents in refrigerator promptly after use.
2.**Wash buffer:** The wash buffer is a 80 × concentrate, please dilute 30 mL with 2370 mL deionized or distilled water (1 part reagent plus 79 parts water) before use.
3.**HRP conjugated RBD:** The HRP-RBD is a 100 × concentrate, please dilute with a 1:99 ratio with HRP dilution buffer. Mix 100 µL of HRP-RBD with 9.9 mL of HRP dilution buffer to make working solution. For 96 tests, 10 mL HRP-RBD working solution is needed.
4.**Samples and Control:** Add 8 µL of sample or controls in supplied 96-well dilution plate and mix with 72 µL of sample dilution buffer, with the volume ratio of 1:9. Note: The HRP-RBD working solution should be stored at +2°C to +8°C and used within 1 days. The suitable working volume should be prepared according to the number of samples.
5. ELISA incubator or water bath must be set to +37 ± 1°C.

**Pipetting protocol**




Pos.: positive control; Neg.: negative control



**Manual test performance**

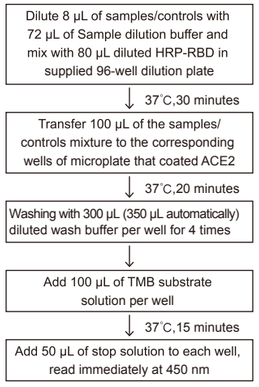
**Neutralization Reaction:**

1.Pipette **80 µL 1 × HRP-RBD working solution** in the separate wells of 96-well dilution plate to mix with the **80 µL of diluted samples or controls** with volume ratio of 1:1.
It's recommended that all Positive and Negative controls should be set in duplicates.
2.Cover the plate with the protective foil and use a microplate shaker to mix the reagents for 60 seconds.
3.Incubate at +37 ± 1°C for 30 minutes.
4.Remove the protective foil and **transfer 100 µL of samples/controls reaction mixture** into the corresponding wells of the microplate wells.
5.Incubate at +37 ± 1°C for 20 minutes.

**Washing:**
6.Remove the protective foil. Empty the wells and subsequently wash **4 times using 300 µL of working-strength wash buffer per well for each wash** (The wash volume for each wash can set as 350 µL per well if the microplate washer is used for washing).
Note: Leave the wash buffer in each well for 30 to 60 seconds per wash cycle. After washing, dispose of all liquid from the microplate by tapping it on paper towel or centrifugation at 1000 × g, 20 seconds to remove all residual wash buffer.
**Substrate incubation:**
7.**Pipette 100 µL of TMB substrate solution** into each well, cover the plate with protective foil and incubate for 15 minutes at +37 ± 1°C (start timing after the addition to the last well).
**Stopping:**
8.Remove the protective foil and pipette **50 µL of stop solution** into each well to stop the reaction.
**Measurement:**
9.Photometric measurement of the color intensity should be made at a **wavelength of 450 nm within 5 minutes of pipetting the stop solution.**



## [PROCEDURE FLOW CHART]




## [AUTOMATICALLY TEST PROTOCOL]

**Neutralization Reaction:**
1.Add **72 µL sample dilution buffer in the wells of 96-well dilution plate.**
2.Follow the pipetting protocol, and add **8 µL samples or controls** into the corresponding wells.
3.Pipette **80 µL 1 × HRP-RBD working solution** in each wells of 96-well dilution plate to mix with the **80 µL of diluted samples or controls** with volume ratio of 1:1.
It's recommended that all Positive and Negative controls should be set in duplicates.
4.Gently shake and incubate at +37 ± 1°C for 50 minutes on shaking incubator.
5.After incubation, **transfer 100 µL of samples/controls reaction mixture** from the dilution plate into the corresponding wells of the microplate wells.
6.Incubate at +37 ± 1°C for 20 minutes.



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**Washing:**
7. Wash the microplate **4 times using 350 µL of working strength wash buffer** for each wash.
Note: Leave the wash buffer in each well for 30 to 60 seconds per wash cycle.
**Substrate incubation:**
8.**Pipette 100 µL of TMB substrate solution** into each well, and incubate for 15 minutes at +37 ± 1°C .
**Stopping:**
9.Pipette **50 µL of stop solution** into each well to stop the reaction.
10.Shake the microplates for 60 seconds to mix the reaction mixture well.
**Measurement:**
11.Photometric measurement of the color intensity shouldbe made at a **wavelength of 450 nm.**

## [QUALITY CONTROL]

For each assay, both Positive and Negative controls must be included to validate the results. The OD450 of each control must meet the requirements as follows, otherwise, the test is invalid and should be repeated.
OD of the Negative control >1.0
OD of the Positive control <0.3

## [INTERPRETING TEST RESULTS]

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:

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

Interpretation of Results:

Inhibition ≥20%: Positive
<20%: Negative

## [LIMITATIONS OF TEST METHODS]

1.For the suspicious samples near borderline. It is recommended to re-determine and supervise dynamically.
2.Due to methodological or immuno-specific reasons, using reagents from different manufacturers to test the same sample may get different test results. Therefore, the test results of different reagents should not be directly compared with each other to avoid erroneous medical interpretations. It is recommend-ed that the laboratory shall indicate the source of the reagents used in the test report. In continuous monitoring, if the reagent type is changed, additional continuity testing should be performed and parallel comparison with the original reagent results to re-determine the baseline value.
3.The test results of this kit are for clinical reference only, and should not be used as the sole basis, and should be combined with other test methods, as live virus and pseudovirus neutralizing antibodies assay.
4.Cross-contamination, microbial contamination, severe hemolysis, or turbid samples may cause incorrect results, try to avoid using such samples.

## [PRODUCT PERFORMANCE INDICATORS]

1.Repeatability: the 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.The cut-off value was determined at 99% sensitivity of 1735 healthy people sera, as <20%.

## [PRECAUTIONS]

1.Read the instruction manual carefully before operation, and perform the test operation strictly following the instruction.
2.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.
3.After the microplate package is opened, the remaining strips should be sealed in an aluminum foil ziplock bag to prevent moisture.
4.The pipette tips cannot be repeatedly used to avoid cross-contamination.
5.Wash the wells gently when adding wash buffer to avoid the contamination between adjacent wells.
6.Residual liquid (>10 μL) in the reagent wells after washing can interfere with the substrate and lead to false low OD readings.
7.The kit contains potential contaminants. Don't handle reagents and samples with bare hands. All samples and used kits should be considered as potentially infectious materials, the dispose of them should under local government and relevant national regulations.
8.Do not use kit past its expiration date.
9.Do not mix components from different kit lots.
10.Do not reuse the used kit.



## [BASIC INFORMATION]

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## [APPROVAL DATE& MODIFICATION DATE OF INSTRUCTION FOR USE]

February 1, 2021





## [Symbols]

| | |
|---|---|
| EC REP | Authorized Representative In the European Community |
| IVD | For in vitro diagnostic use only |
| | Stored at 4 ~ 8℃ |
| | Production Date |
| | Tests per kit |

| | |
|---|---|
| REF | Catalog # |
| LOT | Batch Code |
| | Do not reuse |
| | Do not use if package damaged |

| | |
|---|---|
| | Manufacturer |
| | Expire Date |
| | Consult instructions for use |
| CE | CE Mark |

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