N041-EN.01

Anti-SARS-CoV-2 (B.1.617.2) Neutralizing Antibody Titer Serologic Assay Kit (Spike Trimer)

Pack Size: 96 tests

Catalog Number： RAS-N041

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not for Use in Diagnostic and Therapeutic Applications

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INTENDED USE

This kit is developed for qualitative detection or titer measurement of Anti-SARS-CoV-2 (B.1.617.2）neutralizing antibody（Spike Trimer）in human serum. It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

This kit is developed for detecting neutralizing antibody against SARS-CoV-2 Spike Protein with T19R, G142D, EF156-157del, R158G, L452R, T478K, D614G, P681R, D950N mutation in the sample through a competitive ELISA. The microplate in the kit is pre-coated with Human ACE2 protein. To initiate the experiment, serum samples, Positive control and Negative Control are added to the wells followed by addition of HRP-SARS-CoV-2 Spike Protein. After incubation, the wells are washed and Substrate Solution is added to the wells. The reaction is terminated by the addition of Stop Solution and the intensity of color is measured at 450 nm. The neutralizing antibodies in the samples will compete with ACE2 for HRP-SARS-CoV-2 Spike Protein binding. The intensity of assay signal decrease proportionally with the concentration of Anti-SARS-CoV-2 neutralizing antibodies.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Amount (96 tests)	Format	Storage	
				Unopened	Opened
RAS041-C01	Pre-coated Human ACE2 Microplate	1 plate	Solid	2-8°C	2-8°C
RAS041-C02	Positive Control	100 µL	Liquid	2-8°C	2-8°C
RAS041-C03	Negative Control	100 µL	Liquid	2-8°C	2-8°C
RAS041-C04	HRP-SARS-CoV-2 Spike Protein (B.1.617.2)	15 µg	Powder	2-8°C, avoid light	-70°C, avoid light
RAS041-C05	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS041-C06	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS041-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS041-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or dual wavelength microplate reader with 450 nm filter;

Centrifuge;

37 ºC Incubator;

Single channel or multichannel pipettes with 10 µL, 200 µL and 1000 µL precision;

10 µL, 200 µL and 1000 µL pipette tips;

Test Tubes;

Graduated cylinder;

Deionized or distilled water for dilution;

SPECIMEN COLLECTION AND STORAGE

Use a vacutainer blood collection tube to collect human serum and allow the sample to settle for at least 30 min at room temperature. Then centrifuge for 5 min at 3000 g and use the supernatant for the assay. Run the assay immediately, otherwise store the aliquot below -20°C. Avoidrepeated freeze-thaw cycles.

Note:

a. Hemolysis affects the final detection result, so hemolytic samples are not suitable for this test.

b. No detection method has been established for human plasma or whole blood samples. It is recommended that users establish their own test methods according to their needs.

STORAGE

The unopened kit is stable for 12 months from the date of manufacture if stored at 2°C to 8°C.

The opened kit should be stored per TABLE 1. The shelf life is 30 days from the date of opening.

Note:

a. Do not use reagents past their expiration date.

b. Find the expiration date on the outside packaging.

EAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20℃-25℃) before use.

2. As recommended in Table 2, the lyophilized materials of HRP-SARS-COV-2 Spike Protein(B.1.617.2) will be diluted into a rehydrated solution with ultrapure water/deionized water. Before use, the rehydrated solution needs to be balanced at room temperature of 30 min, shake gently every 10 min. Do not shake or vortex violently. The rehydrated solution should be stored at -70℃, Do not thaw and freeze more than 3 times.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

Catalog	Components	Amount	Stock Solution Con.	Reconstitution Buffer and Vol.
RAS041-C04	HRP-SARS-CoV-2 Spike Protein (B.1.617.2)	15 μg	100 μg/mL	150μL

RECOMMENDED SAMPLE PREPARATION

1. Working fluid preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of HRP-SARS-CoV-2 Spike Protein(B.1.617.2) working fluid:

Dilute HRP-SARS-CoV-2 Spike Protein(B.1.617.2) rehydrated solution to 1.0 μg/ml with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

1.3 Preparation of Positive Control and Negative Control working fluid and pre-treatment of samples:

a. For qualitative detection of antibodies:

Dilute the samples, Positive Control and Negative Control at 1:10 with Dilution Buffer.

b. For determination of antibody titer:

It is recommended to dilute the samples, Positive Control and Negative Control from 1:10-1:320 with Dilution Buffer.

2. Plate set up

Number the diluted samples corresponding to the wells of the Pre-coated Human ACE2 Microplate. Each experiment requires a set of Positive Control and Negative Control working fluid.

3. Add samples

Add 50 μL diluted sample, Positive Control and Negative Control working fluid to the corresponding wells, then add 50 μL HRP-SARS-CoV-2 Spike Protein(B.1.617.2) working fluid to each well. Shake gently to mix.

Note: This step needs to be operated continuously without a long interval to not to affect the results.

4. Incubation

Incubate the plate for 1.0 h at 37°C, Avoid light.

5. Washing

Remove the solution from the wells by aspiration. Add 300 μL 1 x Washing Buffer to each well, gently shake the plate for 30 s. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against paper towels. Repeat the steps above for three times.

6. Substrate Reaction

Add 100 μL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at 37℃ for 20 min. Avoid light.

7. Termination

Add 50 μL **Stop Solution** to each well, shake gently to mix.

Note: *the color in the wells should change from blue to yellow.*

8. Data Recording

Read the absorbance at 450 nm using UV/Vis microplate spectrophotometer.

***Note**: To reduce the background noise, subtract the value read at $OD_{450\ nm}$ with the value read at $OD_{630\ nm}$.*

CUT-OFF VALUE IDENTIFICATION

1. Cut-off value =20% signal inhibition.

$$\text{Percent inhibition} = \left(1 - \frac{OD_{450\ nm}\ \text{of sample}}{OD_{450nm}\ \text{of negative control}}\right) \times 100\%$$

Note：The cut-off value can be determined by the end user.

2. Normal range of Negative control: Negative Control working fluid $OD_{450\ nm} > 0.8$

3. Normal range of Positive control: 1: 10 diluted Positive Control working fluid $OD_{450\ nm} < 0.1$

INTERPRETION OF RESULTS

a. For qualitative detection of antibodies:

Positive reading: Percent inhibition of sample ≥ Cut-off value means Anti-SARS-CoV-2 (B.1.617.2) neutralizing antibody (Spike Trimer) are detected.

Negative reading: Percent inhibition of sample ＜Cut-off value means Anti-SARS-CoV-2 (B.1.617.2) neutralizing antibody (Spike Trimer) are not detected.

b. For determination of antibody titer:

Determination of antibody titer: the positive sample was diluted with a gradient, and the antibody titer of the sample corresponds to the highest dilution factor that still yields a positive reading.

LIMITATIONS OF THE PROCEDURE

This test is designed for detecting human serum of Anti-SARS-CoV-2 (B.1.617.2) neutralizing antibody (Spike Trimer). However, we do not have the LoQ (Limit of Quantitation) and ULMI (upper limit of measuring interval) and cutoff defined for semi-quantitative detection. Interested customer is recommended to establish the semi-quantitative detection procedure themselves.

PERFORMANCE

Precision: Intra batch CV%<15%, Inter batch CV%<15%.

Specificity: 98.8% (one samples show false positive, n=80)

PRECAUSIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. This kit should be used according to the provided instructions.
3. Do not mix reagents from different lots.
4. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in the buffer solution, incubate until the crystals have completely dissolved. Before use, bring the solution back to room temperature.
5. This kit should be stored at 2°C -8°C.
6. Please prepare the working solution of each component according to the needs of the experiment. Except for 1xWashing Buffer, all prepared working solution is for one-time use and cannot be stored.

TYPICAL DATA

a. For qualitative detection of antibodies:

Value Result in units	Result	Test Result Interpretation
Sample Percent inhibition=14%	Negative	Anti-SARS-CoV-2 (B.1.617.2) neutralizing antibody (Spike Trimer) are not detected. No additional test is required.
Sample Percent inhibition=36%	Positive	Anti-SARS-CoV-2 (B.1.617.2) neutralizing antibody (Spike Trimer) are detected. No additional test is required.

b. For determination of antibody titer:

Ratio of Dilution	$OD_{450\ nm}$ (Samples)	Percent inhibition	Result
10	1.101	55%	The titer level of antibody is 20
20	1.849	25%	
40	2.22	10%	
80	2.396	3%	
160	2.444	1%	
blank	2.469	0%	

Note: It is recommended to optimize the dilution ratio of samples to be tested in the experiment. If you want to use a recombinant antibody for quality control, please contact us.