T009-EN.01

Anti-SARS-CoV-2 Total Antibody Titer Serologic Assay Kit (Spike RBD)

Pack Size: 96 tests

Catalog Number： RAS-T009

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

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedures

INTENDED USE

This kit is developed for qualitative detection or titer measurement of Anti-SARS-CoV-2 Total Antibody (Spike RBD) in human serum.It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

The newly identified Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has posed a serious threat to human health. A rapid and effective Assay kit detecting the levels of Anti-SARS-CoV-2 in human serum can facilitate research on characterization of antibodies produced in response to SARS-CoV-2 infection.

This assay kit is used to measure of Anti-SARS-CoV-2 Antibody (Spike RBD) by employing an sandwich ELISA. Immobilize SARS-CoV-2 Spike RBD on the microplate. Then add the samples, incubate and wash the wells. Next add HRP- SARS-CoV-2 Spike RBD to the plate, incubate and wash the wells. Lastly load the substrate into the wells and monitor color development in proportion with the amount of antibody present. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm. The OD Value reflects the amount of antibody bound.

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MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS009-C01	Pre-coated SARS-CoV-2 Spike RBD Microplate	1 plate	Solid	2-8℃	2-8℃
RAS009-C02	Positive Control	200 µL	Liquid	2-8℃	2-8℃
RAS009-C03	Negative Control	200 µL	Liquid	2-8℃	2-8℃
RAS009-C04	HRP-SARS-CoV-2 Spike RBD	10 µg	Powder	2-8℃, avoid light	-70℃, avoid light
RAS009-C05	10xWashing Buffer	50 mL	Liquid	2-8℃	2-8℃
RAS009-C06	Dilution Buffer	50 mL	Liquid	2-8℃	2-8℃
RAS009-C07	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
RAS009-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

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;

SHIPPING AND 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.

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Note:

a. Do not use reagents past their expiration date.

b. Find the expiration date on the outside packaging.

REAGENT PREPARATION

Reconstitute the provided lyophilized materials to stock solutions with distilled, sterile water as recommended in Table 2. solubilize for 15 to 30 min at room temperature with occasional gentle mixing. Avoid vigorous shaking or vertexing. The reconstituted stock solutions should be stored at -70℃. It is recommended to freeze-thaw no more than 3 times. To avoid surface adsorption, loss and inactivation, the reconstituted protein must NOT be aliquoted to less than 5 μg per vial.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

Catalog	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
RAS009-C04	HRP-SARS-CoV-2 Spike RBD	10 μg	100 μg/mL	100 μL water

All reagents should be balanced to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, warm to room temperature until the crystals have completely dissolved.

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 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 -1:640 with Dilution

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Buffer.

1.3 Preparation of HRP-SARS-CoV-2 Spike RBD working fluid:

Dilute HRP-SARS-CoV-2 Spike RBD rehydrated solution to 0.1 μg/ml with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

2. Plate set up

Number the diluted samples corresponding to the wells of the Pre-coated with SARS-CoV-2 Spike RBD Microplate. Each experiment requires a set of Positive Control and Negative Control working fluid.

3. Add Samples

Add 100 μL diluted sample, Positive Control and Negative Control working fluid to the corresponding wells. Seal the plate with microplate sealing film and incubate at 37℃ for 1.0 h.

4. Washing

Remove the remaining solution by aspiration, add 300 μL of 1×Washing Buffer to each well, gently tap the plate for 30 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

5. Add HRP- SARS-CoV-2 Spike RBD

The prepared working fluid should be stored away from light. For all wells, add 100 μL HRP- SARS-CoV-2 Spike RBD working solution. Seal the plate with microplate sealing film and incubate at 37℃ for 1.0 h, avoid light.

6. Washing

Repeat step 4.

7. 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.

8. Termination

Add 50 μL **Stop Solution** to each well, and tap the plate gently for 3 min to allow thorough mixing.

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

9. Data Recording

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

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

CUT-OFF VALUE IDENTIFICATION

Cut-off value =0.1.

Normal range of Negative control: $OD_{450\ nm}$＜0.1, Normal range of Positive control: $OD_{450\ nm}$≥0.5

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

INTERPRETION OF RESULTS

a. For qualitative detection of antibodies:

Positive reading: Percent inhibition of sample ≥ Cut-off value means Anti-SARS-CoV-2 Antibody (Spike RBD) are detected.

Negative reading: Percent inhibition of sample < Cut-off value means Anti-SARS-CoV-2 Antibody (Spike RBD) 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 kit is developed for detecting human serum of Anti-SARS-CoV-2 Antibody(Spike RBD) . 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.

PRECAUTIONS

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.

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4. All reagents should be balanced 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 10x Washing Buffer, all prepared working solution is for one-time use and cannot be stored.

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