T134-EN.01

Human Anti-SARS-CoV-2 (BA.5) Antibody IgG Titer Serologic Assay Kit (Spike RBD)

Pack Size: 96 tests

Catalog Number： RAS-T134

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 product is developed for titer measurement of Anti-SARS-CoV-2(BA.5) Antibody IgG (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 the titer of Anti-SARS-CoV-2 Antibody IgG by employing an indirect ELISA. Immobilize SARS-CoV-2 Spike RBD (BA.5) on the microplate. Then add the samples, incubate and wash the wells. Next add Secondary antibody HRP-Anti-Human IgG 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 color can be measured at 450 nm and 630 nm. The OD Value reflects the amount of antibody bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS134-C01	Pre-coated SARS-CoV-2 Spike RBD (BA.5) Microplate	1 plate	Solid	2-8°C	2-8°C
RAS134-C02	SARS-CoV-2 Antibody Positive Control	100 µL	Liquid	2-8°C	2-8°C
RAS134-C03	SARS-CoV-2 Antibody Negative Control	100 µL	Liquid	2-8°C	2-8°C
RAS134-C04	HRP-Anti-Human IgG	100 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS134-C05	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS134-C06	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS134-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light

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RAS134-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or dual wavelength microplate reader with 450nm and 630nm 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;

STORAGE AND EXPIRY DATE

Unopened kit should be stored at 2℃-8℃ upon receiving.

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.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37 ºC incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

RECOMMENDED PROTOCOL

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-anti-Human IgG** working fluid:

Dilute **HRP-anti-Human IgG** at 1:1000 with Dilution Buffer. The prepared working fluid should avoid light.

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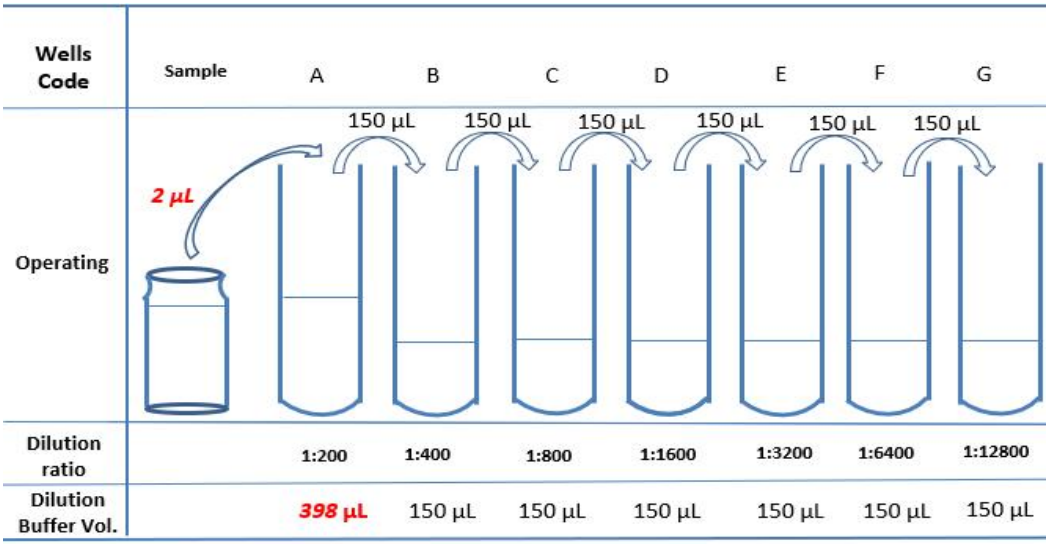
Please prepare it for one-time use only.

2. Add Samples

2.1 Pre-treatment of samples:

Please refer to Table 2 for dilution methods，dilute the sample from 1:200 to 1:12800 using an Dilution Buffer. If the sample to be tested exceeds the recommended dilution range, it is recommended to increase the dilution factor and re-test. Please adjust the specific dosage according to the experimental scheme.

TABLE 2. DILUTION METHOD



2.2 Preparation of Positive Control and Negative Control working fluid:

Dilute the Positive Control at 1:1600 and Negative Control at 1:200 with Dilution Buffer.

2.3 Add Samples and Positive Control and Negative Control:

Please refer to Table 3 for arrangement, add 100 µL Samples and Positive Control and Negative Control to each well. It is recommended that the samples to be tested, Positive Control and Negative Control be added with multiple holes.

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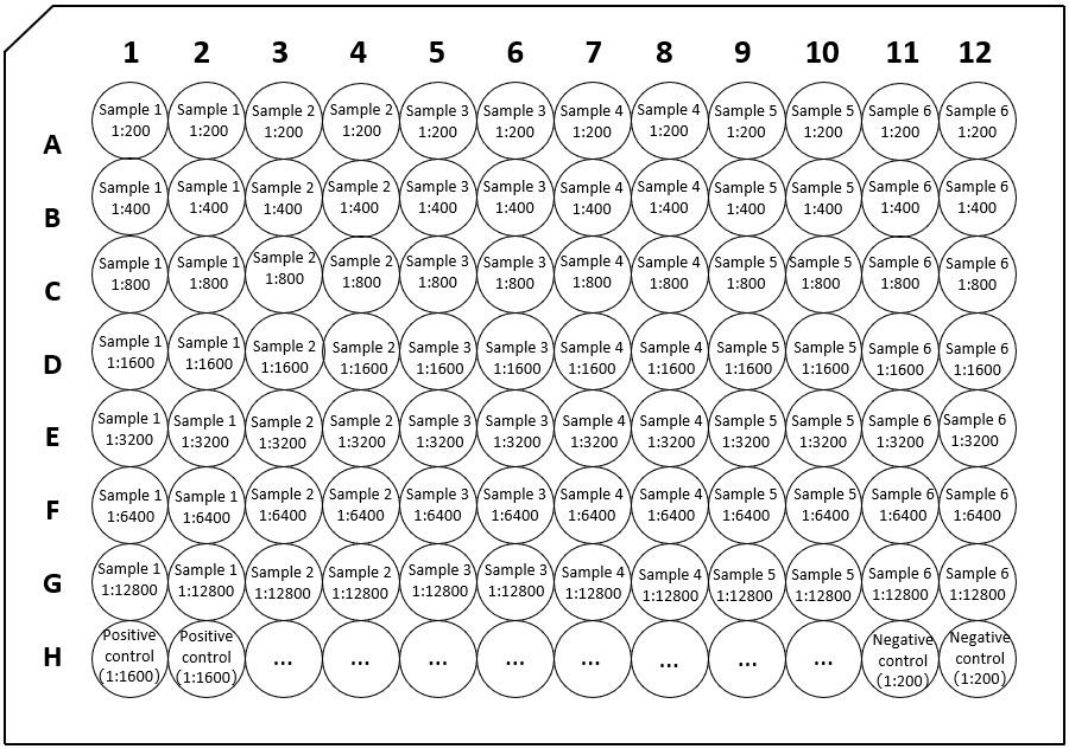

3. Incubation

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

4. 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.

5. Add HRP-anti-human IgG working fluid

Add 100 µL HRP-anti-human IgG working fluid to the corresponding wells, and incubate the plate for 1.0 h at 37°C, 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.

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8. 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.*

9. Data Recording

Read the absorbance at 450nm and 630nm using UV/Vis microplate spectrophotometer.

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

10. Data Analysis

Please analyze the OD value of the reading results according to the instructions of the kit. If the samples or Calibrators are added to several wells, it's necessary to calculate the average value of OD value before data analysis.

CUT-OFF VALUE IDENTIFICATION

Cut-off value =0.1

Normal range of Negative control (1:100): $OD_{450\ nm}$-$OD_{630\ nm}$＜0.1

Normal range of Positive control (1:1600): $OD_{450\ nm}$-$OD_{630\ nm}$≥1.5

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

INTERPRETION OF RESULTS

For 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 IgG (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℃-8℃.
6. Please prepare the working solution of each component according to the needs of the experiment. Except for 1x Washing Buffer, all prepared working solution is for one-time use and cannot be stored.

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