T001-EN.03

Anti-SARS-CoV-2 Antibody IgG Titer Serologic Assay Kit (Spike S1)

Pack Size: 96 tests

Catalog Number： RAS-T001

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 for titer measurement of Anti-SARS-CoV-2 Antibody IgG (Spike S1) 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 S1 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 absorbance can be measured at 450 nm/630 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
RAS001-C01	Pre-coated SARS-CoV-2 Spike S1 Microplate	1 plate	Solid	2-8℃	2-8℃
RAS001-C02	Positive Control	100 µL	Liquid	2-8℃	2-8℃
RAS001-C03	Negative Control	100 µL	Liquid	2-8℃	2-8℃
RAS001-C04	HRP-Anti-Human IgG	200 µL	Liquid	2-8℃,avoid light	2-8℃,avoid light
RAS001-C05	10xWashing Buffer	50 mL	Liquid	2-8℃	2-8℃
RAS001-C06	Dilution Buffer	50 mL	Liquid	2-8℃	2-8℃
RAS001-C07	Substrate Solution	12 mL	Liquid	2-8℃,avoid light	2-8℃,avoid light
RAS001-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Dual wavelength microplate reader with 450 nm/630 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 blood 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

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immediately, otherwise store the aliquot below -20°C. Avoid repeated freeze-thaw cycles.

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.

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 ℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

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-Anti-Human IgG working fluid:

Dilute HRP-Anti-Human IgG at 1:100 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:

It is recommended to dilute the samples and Negative Control from 1:400-1:12800 with Dilution Buffer.

Positive control has a high concentration,it is recommended to dilute the Positive Control from 1:6400-1:409600 with

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Dilution Buffer.

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.Abb HRP-Anti-Human IgG

For all wells, add 100 µL HRP-Anti-Human IgG 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

Read the absorbance at 450 nm/630 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.

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

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Negative control at 1:400 dilution: $OD_{450\ nm}$-$OD_{630\ nm}$＜0.1,

Positive control at 1:6400 dilution:$OD_{450\ nm}$-$OD_{630\ nm}$≥1.0

Note：If $OD_{450\ nm}$-$OD_{630\ nm}$ ***values of controls do not meet the requirement, the test is invalid and must be repeated.***

INTERPRETION OF RESULTS

Positive reading: $OD_{450\ nm}$-$OD_{630\ nm}$ of sample ≥ Cut-off value means Anti-SARS-CoV-2 Antibody IgG (Spike S1) are detected.

Negative reading: $OD_{450\ nm}$-$OD_{630\ nm}$ of sample ＜ Cut-off value means Anti-SARS-CoV-2 Antibody IgG (Spike S1) are not detected.

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 Antibody IgG (Spike S1). 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.The kit cannot be used for quantitative detection.

PERFORMANCE

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

Specificity: 93.8% (five samples show false positive (n=80).

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.
4. All reagents should be warmed to room temperature (20℃-25℃) before use. If crystals have formed in the buffer

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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.

TYPICAL DATA

For determination of antibody titer:

Ratio of Dilution	$OD_{450\ nm}$-$OD_{630\ nm}$(Samples)	Result
400	3.452	The titer level of antibody is 409600
800	3.451	
1600	3.335	
3200	3.054	
6400	2.745	
12800	1.997	
25600	1.168	
51200	0.643	
102400	0.355	
204800	0.188	
409600	**0.103**	
819200	0.058	
Blank	0.035	

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