T070-EN.01

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

Pack Size: 96 tests

Catalog Number： RAS-T070

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

For Research Use Only. Not for Use In Diagnostic Or Therapeutic Procedure

INTENDED USE

This kit is developed for detecting Anti-SARS-CoV-2 (B.1.617.2) Antibody IgG (Spike Trimer) in mouse serum samples.

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 mouse 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 Trimer on the microplate. Then add the samples, incubate and wash the wells. Next add Secondary antibody HRP-Anti-Mouse 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 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
RAS070-C01	Pre-coated SARS-CoV-2 Spike Trimer (B.1.617.2) Microplate	1 plate	Solid	2-8°C	2-8°C
RAS070-C02	Positive Control	100 µL	Liquid	2-8°C	2-8°C
RAS070-C03	Negative Control	100 µL	Liquid	2-8°C	2-8°C
RAS070-C04	HRP-Goat anti-Mouse IgG	10 µg	Powder	2-8°C, avoid light	-70°C, avoid light
RAS070-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS070-C06	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS070-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS070-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

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STORAGE AND VALIDITY INSTRUCTIONS

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.

MATERIALS REQUIRED BUT NOT PROVIDED

Single or dual wavelength microplate reader with 450 nm and 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;

REAGENT 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-Goat anti-Mouse IgG 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	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
RAS070-C04	HRP-Goat anti-Mouse IgG	10 µg	50 µg/mL	200 µL water

RECOMMENDED SAMPLE PREPARATION

1. Working fluid preparation

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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 1:100 with Dilution Buffer.

b. For determination of antibody titer:

It is recommended to dilute the samples, Positive Control and Negative Control from 1:100 -1:25600 with Dilution Buffer.

2. Plate set up

Number the diluted samples corresponding to the wells of the Pre-coated SARS-CoV-2 Spike Trimer (B.1.617.2) 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 1 min, 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-Goat anti-Mouse IgG

Dilute HRP-Goat anti-Mouse IgG stock solution (50 µg/mL) to 0.08 µg/mL with Dilution Buffer to make a working solution. The prepared working fluid should be stored away from light. For all wells, add 100 µL HRP-Goat anti-Mouse 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

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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 and 630 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

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 (1:800): $OD_{450\ nm}$-$OD_{630\ nm}$≥1.6

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

INTERPRETION OF RESULTS

Positive reading: $OD_{450\ nm}$-$OD_{630\ nm}$ of sample ≥ Cut-off value means Anti-SARS-CoV-2(B.1.617.2) Antibody IgG (Spike Trimer) are detected.

Negative reading: $OD_{450\ nm}$-$OD_{630\ nm}$ of sample ＜ Cut-off value means Anti-SARS-CoV-2(B.1.617.2) Antibody IgG (Spike Trimer) 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 mouse serum of Anti-SARS-CoV-2(B.1.617.2) Antibody IgG (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. The kit cannot be used for quantitative detection.

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.

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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 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

Note: The Typical data is for reference only.

a. For qualitative detection of antibodies:

Value Result in units	Result	Test Result Interpretation
$OD_{450\ nm}$ - $OD_{630\ nm}$ =0.041	Negative	Anti-SARS-CoV-2(B.1.617.2) Antibody IgG (Spike Trimer) are not detected. No additional test is required.
$OD_{450\ nm}$ - $OD_{630\ nm}$ =0.592	Positive	Anti-SARS-CoV-2(B.1.617.2) Antibody IgG (Spike Trimer) are detected. No additional test is required.

b. For determination of antibody titer:

Note: *Quality control data between different plates should not be mixed, and negative and positive controls should be set for each test.*

Ratio of Dilution	$OD_{450\ nm}$ - $OD_{630\ nm}$ (Samples)	Result
100	3.433	The titer level of antibody is 25600
200	3.208	
400	2.977	
800	2.357	
1600	1.365	
3200	0.759	
6400	0.401	
12800	0.238	
25600	**0.131**	
blank	0.024	

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