T023-EN.02

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

Pack Size: 96 tests

Catalog Number： RAS-T023

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 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 Protein on the microplate. Then add the samples, incubate and wash the wells. Next add Secondary antibody HRP-Conjugated Antibody 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
RAS023-C01	Pre-coated with SARS-CoV-2 Spike Protein Microplate	1 plate	Solid	2-8°C	2-8°C
RAS023-C02	Anti-SARS-CoV-2 Antibody (Control, Mouse IgG)	100 µL	Liquid	2-8°C	2-8°C
RAS023-C03	HRP-Conjugated Antibody	10 µg	Powder	2-8°C,avoid light	-70°C,avoid light
RAS023-C04	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS023-C05	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS023-C06	Substrate Solution	12 mL	Liquid	2-8°C,avoid light	2-8°C,avoid light
RAS023-C07	Stop Solution	7 mL	Liquid	2-8°C	2-8°C
RAS023-C08	SARS-CoV-2 Antibody Positive Control	100 µL	Liquid	2-8°C	2-8°C
RAS023-C09	SARS-CoV-2 Antibody Negative Control	100 µL	Liquid	2-8°C	2-8°C

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

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Single or 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;

STORAGE

1. Unopened kit should be stored at 2℃-8℃ upon receiving.
2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.
3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

REAGENT PREPARATION

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

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.
RAS023-C03	HRP-Conjugated Antibody	10 µg	50 µg/mL	200 µL water

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:

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Dilute the samples,Positive Control and Negative Control at 1:100 with Dilution Buffer.

b. For semi-quantitative detection or titer measurement of antibodies:

b.1 It is recommended dilute the samples from 1:100 -1:10000 with Dilution Buffer.

b.2 If titer measurement,it is recommended to dilute the samples,Positive Control and Negative Control from 1:100 -1:10000 with Dilution Buffer.

If semi-quantitative detection,it is recommended dilute the Anti-SARS-CoV-2 Antibody (Control, Mouse IgG) from 0.2-6.25 ng/mL with Dilution Buffer.The concentration of Anti-SARS-CoV-2 Antibody (Control, Mouse IgG) is 100 μg/mL.

2. Add Samples

a. For qualitative detection or titer measurement of antibodies:

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

b. For semi-quantitative detection of antibodies:

Add 100 μL diluted sample and Anti-SARS-CoV-2 Antibody (Control, Mouse IgG)(0.2-6.25 ng/mL). Add 100 μL Dilution Buffer to blank control.Seal the plate with microplate sealing film and incubate at 37℃ for 1.0 h.

3. 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.

4. HRP-Conjugated Antibody

Dilute HRP-Conjugated Antibody 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-Conjugated Antibody working solution.Seal the plate with microplate sealing film and incubate at 37℃ for 1.0 h, avoid light.

5. Washing

Repeat step 3.

6. Substrate Reaction

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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, and tap the plate gently for 3 min to allow thorough mixing.

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

8. Data Recording

Read the absorbance at 450 nm and 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.

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

Normal range of Positive control: $OD_{450\ nm}$-$OD_{630\ nm}$≥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 IgG (Spike Trimer) are detected.

Negative reading: Percent inhibition of sample ＜Cut-off value means Anti-SARS-CoV-2 Antibody IgG (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.

c. For semi-quantitative detection of antibodies:

To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is subtracted to the OD value of the blank control. The standard curve is plotted with the standard concentration as x-axis andthe calibrated absorbance value as y-axis. Four parameters logistic or other statistical software are used to draw the

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standard curve and calculate the sample concentration.

Note:The results are for reference only.

LIMITATIONS OF THE PROCEDURE

This test is designed for detecting mouse serum of Anti-SARS-CoV-2 Antibody IgG (Spike Trimer).

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. 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, all prepared working solution is for one-time use and cannot be stored.

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