T019-EN.01

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

Pack Size: 96 tests

Catalog Number： RAS-T019

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 semi-quantitative detection or titer measurement of Anti-SARS-CoV-2 Antibody IgG (Spike RBD) in monkey 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 monkey serum can facilitate research on characterization of antibodies produced in response to SARS-CoV-2 infection.

This assay kit is developed for semi-quantitative detection or titer measurement of Anti-SARS-CoV-2 Antibody IgG (Spike RBD) by employing an indirect ELISA. Immobilize SARS-CoV-2 Spike RBD on the microplate. Then add the samples, incubate and wash the wells. Next add Secondary antibody HRP-Goat anti-Monkey 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. The OD Value reflects the amount of antibody bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS019-C01	Pre-coated SARS-CoV-2 Spike RBD Microplate	1 plate	Solid	2-8℃	2-8℃
RAS019-C02	Anti-SARS-CoV-2 Antibody (Control, Monkey IgG)	100 µL	Liquid	2-8℃	2-8℃
RAS019-C03	Positive Control	100 µL	Liquid	2-8℃	2-8℃
RAS019-C04	Negative Control	100 µL	Liquid	2-8℃	2-8℃
RAS019-C05	HRP-Goat anti-Monkey IgG	100 µL	Liquid	2-8℃,avoid light	2-8℃,avoid light
RAS019-C06	10×Washing Buffer	50 mL	Liquid	2-8℃	2-8℃
RAS019-C07	Dilution Buffer	50 mL	Liquid	2-8℃	2-8℃
RAS019-C08	Substrate Solution	12 mL	Liquid	2-8℃,avoid light	2-8℃,avoid light
RAS019-C09	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

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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;

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, warm until the crystals have completely dissolved and bring 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 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 1:50 with Dilution Buffer.

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

It is recommended dilute the Anti-SARS-CoV-2 Antibody (Control, Monkey IgG) from 0.98-125 ng/ml with Dilution Buffer. Please refer to the tube label for concentration.

It is recommended to dilute the samples,Positive Control and Negative Control from 1:50-1:1600 with Dilution Buffer.

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. Add 100 µL Dilution Buffer to blank control.Seal the plate with microplate sealing film and incubate at 37°C 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. HRP-Goat anti-Monkey IgG

Dilute HRP-Goat anti-Monkey IgG stock solution at 1:1000 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- Monkey IgG working solution.Seal the plate with microplate sealing film and incubate at 37°C 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°C for 20 min, avoid light.

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8. Termination

Add 50 μL **Stop Solution** to each well, and tap the plate gently for 1 to 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 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 IgG (Spike RBD) are detected.

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

c. For semi-quantitative detection of antibodies:

If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated. To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is subtracted from the OD value of the blank control. The standard curve is plotted with the standard

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concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic or other statistical software are used to draw the standard curve and calculate the sample concentration.

LIMITATIONS OF THE PROCEDURE

This kit is developed for detecting monkey serum of Anti-SARS-CoV-2 Antibody IgG (Spike RBD).

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 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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TYPICAL DATA

a. For qualitative detection of antibodies:

Value Result in	Result	Test Result Interpretation
$OD_{450\ nm}$=0.065	Negative	Anti-SARS-CoV-2 Antibody IgG (Spike RBD) are not detected. No additional test is required.
$OD_{450\ nm}$=0.590	Positive	Anti-SARS-CoV-2 Antibody IgG (Spike RBD) are detected. No additional test is required.

b.For determination of antibody titer:

Note: It is recommended to optimize the dilution ratio of samples to be tested in the experiment. If you want to use a recombinant antibody for quality control, please contact us.

Ratio of Dilution	$OD_{450\ nm}$-$OD_{630\ nm}$(Samples)	Result
50	2.792	The titer level of antibody is 25600
100	2.791	
200	2.754	
400	2.559	
800	2.224	
1600	1.611	
3200	0.988	
6400	0.609	
12800	0.300	
25600	**0.158**	
51200	0.079	
102400	0.046	
blank	0.011	

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