Anti-SARS-CoV-2 Antibody IgG1 Titer Serologic Assay kit

Pack Size: 96 tests

Catalog Number： RAS-T014

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 serologic test for IgG1 titer of Anti-SARS-CoV-2 antibody in serum/plasma in vitro.

It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

The newly identified Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) poses continual threat to human health due to rapid transmission worldwide. Unprecedented vaccination campaigns have now begun with multiple candidates. To support these studies, ACRO has developed Anti-SARS-CoV-2 subtype antibody Serologic Assay kit including: IgG1, IgG2, IgG3, IgG4.

This assay kit is used to measure the levels of Anti-SARS-CoV-2 antibody IgG1 by employing an indirect ELISA format. The microplate in the kit has been pre-coated with SARS-CoV-2 Spike RBD. First add the diluted samples to the plate and wash the wells after incubation. Afterwards add HRP-Mouse anti-Human IgG1 to the plate and wash the wells after incubation. Lastly the substrate is loaded into the wells and color develops in proportion to the amount of antibody. The reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450 nm. The OD Value reflects the amount of antibody bound.

US and Canada: **Tel**: +1 800-810-0816
Asia and Pacific: **Tel**: +86 400-682-2521
Web: http://www.acrobiosystems.com
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MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS014-C01	Pre-coated SARS-CoV-2 Spike RBD Microplate	1plate	Solid	2-8℃	2-8℃
RAS014-C02	Anti-SARS-CoV-2 Antibody (Control, IgG1)	100μL	Liquid	2-8℃	2-8℃
RAS014-C03	HRP-Mouse anti-Human IgG1	20μL	Liquid	2-8℃ avoid light	2-8℃ avoid light
RAS014-C04	10×Washing Buffer	50mL	Liquid	2-8℃	2-8℃
RAS014-C05	Dilution Buffer	50mL	Liquid	2-8℃	2-8℃
RAS014-C06	Substrate Solution	12mL	Liquid	2-8℃ avoid light	2-8℃ avoid light
RAS014-C07	Stop Solution	7mL	Liquid	2-8℃	2-8℃

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or dual wavelength microplate reader with 450 nm filter； Centrifuge； 37 ºC Incubator；

10 µL, 200 µL and 1000 µL precision pipettes； 10 µL, 200 µL and 1000 µL pipette tips；

Multichannel pipettes； Tubes； Graduated cylinder to prepare Wash Solution

Deionized or distilled water to dilute 10×Washing Buffer;

PECIMEN COLLECTION AND STORAGE

For human serum, use a blood separator tube and allow sample to clot for 30 minutes at room temperature, then centrifuge for 5 minutes at 3000 g. Run assay immediately, otherwise store aliquot below -20°C. Avoid repeated freeze-thaw cycles.

Note: Hemolysis of sample affects the final detection result, so hemolytic samples are not suitable for this test.

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.

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Note:

a. Do not use reagents past their expiration date.

b. Find the expiration date on the outside packaging.

REAGENT PREPARATION

All reagents should be balance to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, worm to room temperature until the crystals have completely dissolved.

1×Washing Buffer: prepare 500mL 1×Washing buffer by adding 50 mL 10 ×Washing Buffer to 450mL distilled water.

RECOMMENDED PROTOCOL

1. Add Samples

Make series dilution of the tested samples with Dilution Buffer. The recommended dilution of the sample is from 1:50 to 1:6400. Add 100μL serially diluted samples to each well. For Blank Control wells, please add 100 μL Dilution Buffer to the well. Seal the plate with microplate sealing film and incubate at 37℃ for 1 hour. Avoid light.

If the antibody concentration in the sample is analyzed semi-quantitatively, Anti-SARS-CoV-2 Antibody (Control, IgG1) reference provided can be diluted with Dilution Buffer, and the recommended concentration range of dilution is 40-1250 ng / ml.

2. Washing

Remove the remaining solution by aspiration, add 300 μL of 1×Washing Buffer: to each well, gently tap the plate for 1 minute, 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.

3. HRP-Mouse anti-Human IgG1

Dilute **HRP-Mouse anti-Human IgG1** stock solution to 1:2000 with Dilution Buffer to make working solution. For all wells, add 100 μL **HRP-Mouse anti-Human IgG1** working solution, seal the plate with microplate sealing film and incubate at 37℃ for 1 hour, avoid light.

4. Washing

Repeat step 2.

5. 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 minutes, avoid light.

6. Termination

Add 50 μL **Stop Solution** to each well, and tap the plate gently for 3 minutes to allow thorough mixing.

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

7. Data Recording

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

Note*: the plate may be read at 630nm and the signal-to-background ratio may be reduced.*

CUT-OFF VALUE IDENTIFICATION

Cut-off value =0.1.

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

INTERPRETION OF RESULTS

Positive: OD value of sample≥Cut-off value means Anti-SARS-CoV-2 antibody are detected.

Negative: OD value of sample＜Cut-off value means Anti-SARS-CoV-2 antibody are not detected.

CALCULATION OF IgG TITER

The maximum dilution multiple of the positive test results was selected, and the corresponding OD value of the maximum dilution / Cut-off × dilution multiple, the calculated value of was the antibody titer corresponding to the sample.

LIMITATIONS OF THE PROCEDURE

This test is designed for qualitative or semi quantitative detection of Anti-SARS-CoV-2 Antibody IgG1.

LIMITATIONS OF THE PROCEDURE

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

2. The kit should be used according to the instructions.
3. Do not mix reagents from different lots.
4. All reagents should be balance to room temperature(20℃-25℃) before use. If crystals have formed in buffer solution, worm to room temperature until the crystals have completely dissolved.
5. The kit should be stored at 2°C to 8°C.

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