



T017-EN.01

Anti-SARS-CoV-2 Antibody IgG4 Titer Serologic Assay Kit

Pack Size: 96 tests

Catalog Number: RAS-T017

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

For Research Use Only. Not for Use in Diagnostic or Therapeutic Procedures

[HTTP://WWW.ACROBIOSYSTEMS.COM](http://www.acrobiosystems.com)

INTENDED USE

This kit is developed for detecting titer of Anti-SARS-CoV-2 IgG4 antibody in serum in vitro.

It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

The ongoing SARS-COV-2 pandemics has sickened 3.7 million people around the world, claiming over 1,000,000 lives. To deal with this global public health crisis, unprecedented efforts are being made to study the virus, SARS-CoV-2. At present, there are 193 new crown vaccine projects registered with the World Health Organization are being developed. To support these studies, ACRO has developed Anti-SARS-CoV-2 subtype antibody Serologic Assay kits including: IgG1, IgG2, IgG3, IgG4.

This assay kit is used to measure the levels of Anti-SARS-CoV-2 Antibody IgG4 by employing an indirect ELISA. The microplate in the kit has been pre-coated with SARS-CoV-2 Spike RBD. First add the samples to the plate, incubate and wash the wells. Next add HRP-Mouse anti-Human IgG4 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 color 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
RAS017-C01	Pre-coated with SARS-CoV-2 Spike RBD Microplate	1 plate	Solid	2-8°C	2-8°C
RAS017-C02	Anti-SARS-CoV-2 Antibody (Control, IgG4)	10 µg	Powder	2-8°C	-70°C
RAS017-C03	HRP-Mouse anti-Human IgG4	50 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS017-C04	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS017-C05	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS017-C06	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS017-C07	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

SHIPPING AND 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.

MATERIALS REQUIRED BUT NOT PROVIDED

Single or dual wavelength microplate reader with 450 nm and 630 nm filter;

Centrifuge;

37 °C 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. All reagents should be warmed to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, place the sample in an 37 °C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

2. Reconstitute the provided lyophilized materials to stock solutions with distilled, sterile water as recommended in Table 2, Solubilize for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking or vortexing.

The reconstituted stock solutions should be stored at -70°C. **It is recommended not to freeze-thaw 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.
RAS017-C02	Anti-SARS-CoV-2 Antibody (Control, IgG4)	10 µg	100 µg/mL	100 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 500mL.

2. Add Samples

Make series dilution of the tested samples with Dilution Buffer. The recommended dilution of the sample is from 1:50 to 1:10000. 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°C for 1 hour. Avoid light.

If the antibody concentration in the sample is analyzed semi-quantitatively, Anti-SARS-CoV-2 Antibody (Control, IgG4)

reference provided can be diluted with Dilution Buffer, and the recommended concentration range of dilution is

0.098-12.5 ng/mL.

3. Washing

Remove the remaining solution by aspiration, add 300 μ L of 1 \times Washing Buffer to each well, gently tap the plate for 1 minute, remove any remaining 1 \times 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-Mouse anti-Human IgG4

Dilute **HRP-Mouse anti-Human IgG4** at 1:1000 with Dilution Buffer. For all wells, add 100 μ L **HRP-Mouse anti-Human IgG4** working solution, seal the plate with microplate sealing film and incubate at 37°C for 1 hour, avoid light.

5. Washing

Repeat step 3.

6. Substrate Reaction

Add 100 μ L **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at 37°C for 20 min, avoid light.

7. Termination

Add 50 μ L **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, subtract the value read at $OD_{450\text{ nm}}$ with the value read at $OD_{630\text{ nm}}$.

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 \geq 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 \times 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 IgG4.

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°C-25°C) 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.