

# **Anti-SARS-CoV-2 (B.1.1.529) Antibody IgG1 Titer Serologic Assay Kit (Spike RBD)**

**Pack Size: 96 tests**

**Catalog Number: RAS-T065**

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

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

**INTENDED USE**

The kit is developed for qualitative detection or titer measurement of Anti-SARS-CoV-2 (B.1.1.529) antibody IgG1 (Spike RBD) in human serum. It is intended for research use only (RUO).

**PRINCIPLE OF THE ASSAY**

The ongoing SARS-COV-2 pandemics has sickened 440 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 IgG1 by employing an indirect ELISA. The microplate in the kit has been pre-coated SARS-CoV-2 (B.1.1.529) Spike RBD. First add the samples to the plate, incubate and wash the wells. Next add HRP-Mouse anti-Human IgG1 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<br>(96 tests) | Format | Storage            |                    |
|------------|--|--------------------|--------|--------------------|--------------------|
|            |  |                    |        | Unopened           | Opened             |
| RAS065-C01 | Pre-coated SARS-CoV-2 (B.1.1.529) Spike RBD Microplate | 1 plate            | Solid  | 2-8°C              | 2-8°C              |
| RAS065-C02 | Anti-SARS-CoV-2 Antibody (Control, IgG1)               | 10 µg              | Powder | 2-8°C              | -70°C              |
| RAS065-C03 | HRP-Mouse anti-Human IgG1                              | 50 µL              | Liquid | 2-8°C, avoid light | 2-8°C, avoid light |
| RAS065-C04 | 10×Washing Buffer                                      | 50 mL              | Liquid | 2-8°C              | 2-8°C              |
| RAS065-C05 | Dilution Buffer  | 50 mL              | Liquid | 2-8°C              | 2-8°C              |
| RAS065-C06 | Substrate Solution                                     | 12 mL              | Liquid | 2-8°C, avoid light | 2-8°C, avoid light |
| RAS065-C07 | Stop Solution  | 7 mL               | Liquid | 2-8°C              | 2-8°C              |

## **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. Bring all reagents and samples 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.

*Note:* 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. |
|------------|--|-------|---------------------|--------------------------------|
| RAS065-C02 | Anti-SARS-CoV-2 Antibody (Control, IgG1) | 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 500 mL.

### **2. Add Samples**

Make series dilution of the tested samples with Dilution Buffer. The recommended dilution of the sample is from 1:50 to 1:6400. 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°C for 1.0 h. 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 78-1250 ng/mL.

### **3. Washing**

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, gently tap the plate, 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-Mouse anti-Human IgG1**

Dilute **HRP-Mouse anti-Human IgG1** at 1:2000 with Dilution Buffer. For all wells, add 100 µL **HRP-Mouse anti-Human IgG1** working solution, seal the plate with microplate sealing film and incubate at 37°C for 1.0 h, avoid light.

### **5. Washing**

Repeat step 3.

### **6. 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.

### **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, 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 reading: OD value of sample  $\geq$  Cut-off value means Anti-SARS-CoV-2 antibody are detected.

Negative reading: 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 titer detection of Anti-SARS-CoV-2 (B.1.1.529) Antibody IgG1.

### **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°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 1xWashing Buffer, all prepared working solution is for one-time use and cannot be stored.