

# **Anti-SARS-CoV-2 Antibody IgG Quantitative Detection Kit (Spike RBD)**

**Pack Size: 96 tests**

**Catalog Number: RAS-T049**

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

***For Research Use Only. Not for Use in Diagnostic and Therapeutic Applications***

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**INTENDED USE**

This kit is developed for quantitative detection of Anti-SARS-CoV-2 Antibody IgG (Spike RBD) in human serum. 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 human 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 RBD on the microplate. Then add the samples, incubate and wash the wells. Next add Secondary antibody HRP-Anti-Human 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、630 nm. The OD Value reflects the amount of antibody bound.

**MATERIALS PROVIDED**

**TABLE 1. MATERIALS PROVIDED**

Catalog	Components	Amount (96 tests)	Format	Storage	
				Unopened	Opened
RAS049-C01	Pre-coated SARS-CoV-2 Spike RBD Microplate	1 plate	Solid	2-8°C	2-8°C
RAS049-C02	Calibrator1	0.5 mL	Powder	2-8°C	-70°C
RAS049-C03	Calibrator2	0.5 mL	Powder	2-8°C	-70°C
RAS049-C04	Calibrator3	0.5 mL	Powder	2-8°C	-70°C
RAS049-C05	Calibrator4	0.5 mL	Powder	2-8°C	-70°C
RAS049-C06	Calibrator5	0.5 mL	Powder	2-8°C	-70°C
RAS049-C07	Calibrator6	0.5 mL	Powder	2-8°C	-70°C
RAS049-C08	HRP-Anti-Human IgG	10 µg	Powder	2-8°C, avoid light	-70°C, avoid light
RAS049-C09	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS049-C10	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C

RAS049-C11	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS049-C12	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

**STORAGE**

The unopened kit should be stored at 2°C to 8°C. The expiry date of the kit is 12 months.

The opened kit should be stored per TABLE 1. The shelf life is 1 month from the date of opening.

*Note: a. After reconstitution of lyophilized components should be stored at -70°C. Do not thaw and freeze more than 3 times.*

*b. Do not use reagents past their expiration date.*

**REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED**

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

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;

**SPECIMEN COLLECTION AND STORAGE**

1. Heat Inactivation: Heat inactivate samples by placing in a water bath at 56 °C for 30 min.

*Note: Do not leave samples at 56 °C for longer than 1.0 h.*

2. Bring samples to room temperature (20°C-25°C) before use, shake gently to mix.

3. If samples need to be stored, please store the aliquot below -20°C. Avoid repeated freeze-thaw cycles.

*Note:*

*a. Samples must be heat inactivated prior to use in this assay.*

*b. Hemolysis affects the final detection result, so hemolytic samples are not suitable for this test.*

*c. No detection method has been established for human plasma or whole blood samples. It is recommended that users establish their own test methods according to their needs.*

## **REAGENT PREPARATION**

1. Bring all reagents and samples to room temperature (20°C-25°C) before use.
2. As recommended in Table 2, the lyophilized materials of HRP-Anti-Human IgG and Calibrator (1 to 6) will be diluted into a rehydrated solution with ultrapure water/deionized water. Before use, the rehydrated solution needs to be balanced at room temperature of 30 min, shake gently every 10 min. Do not shake or vortex violently. The rehydrated solution should be stored at -70°C, Do not thaw and freeze more than 3 times.

**TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS**

Catalog	Components	Amount	Stock Solution Con.	Reconstitution Buffer and Vol.
RAS049-C02	Calibrator1	0.5 mL	0.291 BAU/mL	0.5 mL water
RAS049-C03	Calibrator2	0.5 mL	0.137 BAU/mL	0.5 mL water
RAS049-C04	Calibrator3	0.5 mL	0.065 BAU/mL	0.5 mL water
RAS049-C05	Calibrator4	0.5 mL	0.029 BAU/mL	0.5 mL water
RAS049-C06	Calibrator5	0.5 mL	0.010 BAU/mL	0.5 mL water
RAS049-C07	Calibrator6	0.5 mL	0 BAU/mL	0.5 mL water
RAS049-C08	HRP-Anti-Human IgG	10 µg	100 µg/mL	0.1 mL 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 HRP-Anti-Human IgG working fluid:

Dilute HRP-Anti-Human IgG rehydrated solution to 0.04 µg/mL with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

### **2. Add samples and Incubation**

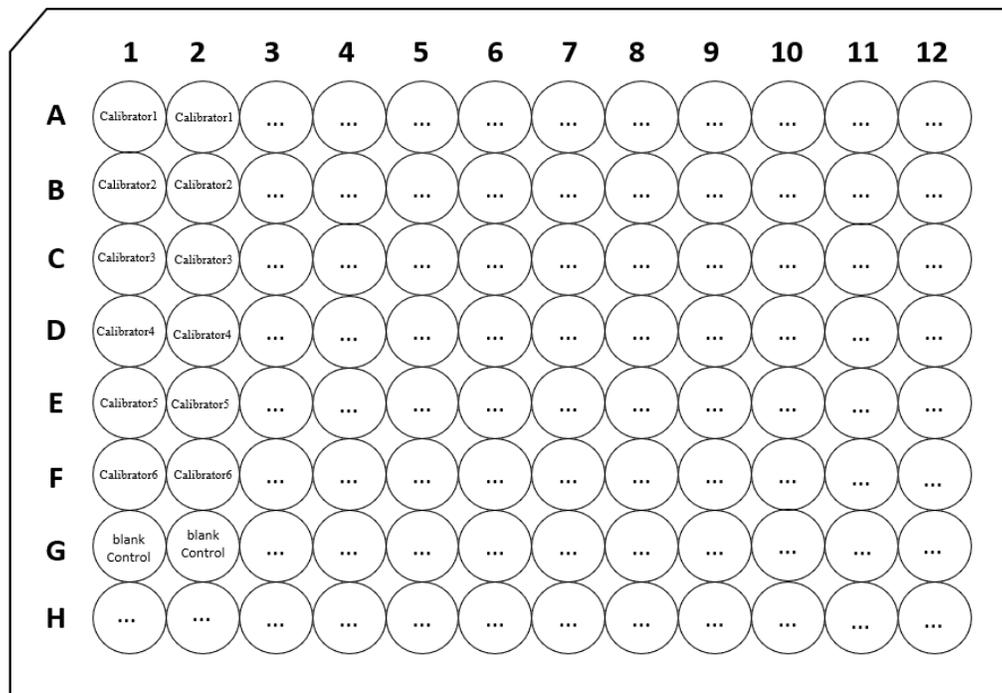
- a. For blank Control wells: Please add 100 µL Dilution Buffer.
- b. For Calibrators: Add 100µL Calibrator1-6 (**Use directly after remelting**) to each well.
- c. For samples: It is recommended to dilute the samples 1:100 with Dilution Buffer, add 100µL to each well.

**Please Seal the plate with microplate sealing film and incubate at 37°C for 1.0 h.**

*Note:*

*It is recommended that at least samples and Calibrators be added to double wells.*

**FIGURE 1. PLATE LAYOUT**



### 3. Washing

Remove the solution from the wells by aspiration. Add 300 µL 1 x Washing Buffer to each well, gently shake the plate for 30 s. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against paper towels. Repeat the steps above for three times.

### 4. Add HRP-anti-Human IgG working fluid

Add 100 µL HRP-anti-Human IgG working fluid to the corresponding wells, and Incubate the plate for 1.0 h at 37°C, Avoid light.

### 5. Washing

Repeat step 3.

## 6. Substrate Reaction

Add 100  $\mu$ 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  $\mu$ L **Stop Solution** to each well, shake gently to mix.

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

## 8. Data Recording

Read the absorbance at 450 nm、630 nm using UV/Vis microplate spectrophotometer.

*Note: To reduce the background noise, subtract the value read at OD<sub>450 nm</sub> with the value read at OD<sub>630 nm</sub>.*

## 9. Data analysis:

a. Please analyze the OD value of the reading results according to the instructions of the kit. If the samples or Calibrators are added to several wells, it's necessary to calculate the average value of OD value before data analysis.

b. Establish a standard curve with linear equation. To calibrate absorbance value obtained by the calibrator curve, the OD value of the sample to be measured is subtracted to the OD value of the blank control. The calibrator curve is plotted with the calibrator concentration as x-axis and the calibrated absorbance value as y-axis. The linear regression equation was used to draw the calibration curve and calculate the concentration of samples.

c. Detection Range: 0.029 BAU/mL-0.291 BAU/mL. The LoQ is 0.029 BAU/mL. Values of samples are greater than the analytical measuring range should be reported as >0.291 BAU/mL or dilute the samples so that it is within the linear range. Values of samples are less than the LOQ should be reported as <0.029 BAU/mL.

## QUALITY CONTROL

Quality standards of Linearity: Correlation coefficient of the Calibrators curve  $R^2 \geq 0.9900$ .

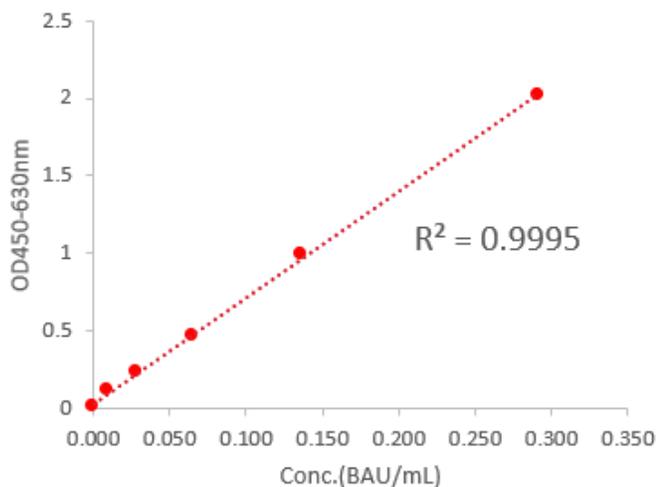
## PRECAUTIONS

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.

**TYPICAL DATA**

The following data is for reference only. The sample concentration was calculated based on the results of the calibrator curve.



(BAU/mL)	O.D. -1	O.D. -2	Average
0.291	2.005	2.023	2.014
0.137	1.042	0.946	0.994
0.065	0.460	0.488	0.474
0.029	0.239	0.231	0.235
0.010	0.115	0.122	0.119
0.000	0.012	0.011	0.012