

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

Pack Size: 96 tests

Catalog Number: RAS-T024

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 product is developed for titer measurement 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 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
RAS024-C01	Pre-coated SARS-CoV-2 Spike RBD Microplate	1 plate	Solid	2-8°C	2-8°C
RAS024-C02	Positive Control	100 µL	Liquid	2-8°C	2-8°C
RAS024-C03	Negative Control	100 µL	Liquid	2-8°C	2-8°C
RAS024-C04	HRP-Anti-Human IgG	200 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS024-C05	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C

RAS024-C06	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS024-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS024-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

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;

SHIPPING AND STORAGE

1. The unopened kit is stable for 12 months from the date of manufacture if stored at 2°C to 8°C.
2. The opened kit should be stored per TABLE 1. The shelf life is 30 days from the date of opening.
3. The kit shipped at room temperature that had been validated. Please contact us if you need blue ice shipping, but additional freight may be followed.

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°C-25°C) before use. If crystals have formed in buffer solution, warm to room temperature until the crystals have completely dissolved.

RECOMMENDED PROTOCOL

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** at 1:99 with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

1.3 Preparation of Positive Control and Negative Control working fluid and pre-treatment of Samples:

It is recommended to dilute the samples, Positive Control and Negative Control from 1:400-1:12800 with Dilution Buffer.

2. Add Samples

Add 100 µL diluted Sample, Positive Control and Negative Control working fluid to the corresponding wells.

3. Incubation

Incubate the plate for 1.0 h at 37°C, Avoid light.

4. 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.

5. 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.

6. Washing

Repeat step 4.

7. 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.

8. Termination

Add 50 μ L **Stop Solution** to each well, shake gently to mix.

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

9. 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

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.

LIMITATIONS OF THE PROCEDURE

This kit is developed for detecting human serum of Anti-SARS-CoV-2 Antibody IgG (Spike RBD). However, we do not have the LoQ (Limit of Quantitation) and ULMI (upper limit of measuring interval) and cutoff defined for semi-quantitative detection. Interested customer is recommended to establish the semi-quantitative detection procedure themselves.

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 1x Washing Buffer, all prepared working solution is for one-time use and cannot be stored.