

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

Pack Size: 96 tests

Catalog Number: AMS.TAS-K002-96tests

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

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedures

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INTENDED USE

The kit is used for the detection of SARS-CoV-2 IgG antibodies in human serum and plasma samples.

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.

Principle of Anti-SARS-CoV-2 Antibody IgG Titer Serologic Assay Kit (Spike Protein RBD) is an indirect ELISA. Immobilize SARS-CoV-2 Spike Protein RBD on the microplate, and then add the samples, until the incubation end wash the wells, the Secondary antibody HRP-Anti-Human IgG is added to the plate, Following a wash, the substrate solution is loaded and color develops in proportion to the amount of antibodies. 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.

MATERIALS PROVIDED**TABLE 1. MATERIALS PROVIDED**

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
TAS002-C01	High-bind Plate	1 plate	Solid	2-8°C	2-8°C
TAS002-C02	SARS-CoV-2 Spike Protein RBD	30 µg	Powder	2-8°C	-70°C
TAS002-C03	Anti-SARS-CoV-2 Antibody (Control, IgG)	10 µg	Powder	2-8°C	-70°C
TAS002-C04	HRP-Anti-Human IgG	10 µg	Powder	2-8°C	-70°C avoid light
TAS002-C05	Coating Buffer	12 mL	Liquid	2-8°C	2-8°C
TAS002-C06	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
TAS002-C07	Blocking / Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
TAS002-C08	Substrate Solution	12 mL	Liquid	2-8°C	2-8°C
TAS002-C09	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

SHIPPING AND STORAGE

This kit is shipped at room temperature.

The unopened kit is stable for at least 1 year from the date of manufacture if stored at 2°C to 8°C, and the opened kit is stable for up to 3 months from the date of opening.

REAGENT PREPARATION

Reconstitute the provided lyophilized materials to stock solutions with 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.

Note: HRP-Anti-Human IgG stock solution should be protected from light.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

Catalog	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
TAS002-C02	SARS-CoV-2 Spike protein RBD	30 µg	200 µg/mL	150 µL water
TAS002-C03	Anti-SARS-CoV-2 Antibody (Control, IgG)	10 µg	100 µg/mL	100 µL water
TAS002-C04	HRP-Anti-Human IgG	10 µg	100 µg/mL	100 µL water

All reagents should be balance to room temperature(20°C-25°C) 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. Coating

Dilute **SARS-CoV-2 Spike protein RBD** stock solution (200 µg/mL) to 2 µg/mL with Coating Buffer to make **SARS-CoV-2 Spike protein RBD** working solution. Add 100 µL of working solution (2 µg/mL) to each well, seal the plate with microplate sealing film and incubate overnight (or 16 hours) at 4°C.

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. Blocking

Add 100 µL Blocking / Dilution Buffer provided to each well, seal the plate with microplate sealing film and incubate at 37°C for 1.5 hours.

4. Washing

Repeat step 2.

5. Add Samples

Make series dilution of the tested samples with Blocking / Dilution Buffer. The recommended dilution of the sample is from 1:400 to 1:10000. Add 100µL serially diluted samples to each well. And 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, the Anti-SARS-CoV-2 Antibody (Control, IgG) reference provided can be diluted with Blocking / Dilution Buffer, and the recommended concentration range of dilution is 0.2-3 ng / ml.

6. Washing

Repeat step 2.

7. HRP-Anti-Human IgG

Dilute **HRP-Anti-Human IgG** stock solution (100 µg/mL) to 0.04 µg/mL with Blocking / Dilution Buffer to make working solution. For all wells, add 100 µL **HRP-Anti-Human IgG** working solution, seal the plate with microplate sealing film and incubate at 37°C for 1 hour, avoid light.

8. Washing

Repeat step 2.

9. Substrate Reaction

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

10. 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.

11. 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.



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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 Spike protein RBD are detected.

Negative: OD value of sample $<$ Cut-off value means Anti-SARS-CoV-2 Spike protein RBD 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 IgG (Spike protein RBD).

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°C-25°C) 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.