T045-EN.02

Mouse Anti-SARS-CoV-2 Antibody IgG Titer Serologic Assay Kit (Spike S1)

Pack Size: 96 tests

Catalog Number： RAS-T045

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

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

INTENDED USE

This kit is developed for detecting Anti-SARS-CoV-2 Antibody IgG (Spike S1) in mouse serum samples.

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 mouse 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 S1 on the microplate. Then add the samples, incubate and wash the wells. Next add Secondary antibody HRP-Conjugated Antibody 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
RAS045-C01	Pre-coated with SARS-CoV-2 Spike S1 Microplate	1 plate	Solid	2-8°C	2-8°C
RAS045-C02	Anti-SARS-CoV-2 Antibody (Control, Mouse IgG)	100 µL	Liquid	2-8°C	2-8°C
RAS045-C03	SARS-CoV-2 Antibody Positive Control	100 µL	Liquid	2-8°C	2-8°C
RAS045-C04	SARS-CoV-2 Antibody Negative Control	100 µL	Liquid	2-8°C	2-8°C
RAS045-C05	HRP-Conjugated Antibody	10 µg	Powder	2-8°C, avoid light	-70°C, avoid light
RAS045-C06	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS045-C07	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

RAS045-C08	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
RAS045-C09	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

STORAGE AND VALIDITY INSTRUCTIONS

1. Unopened kit should be stored at 2℃-8℃ upon receiving.

2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.

3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

MATERIALS REQUIRED BUT NOT PROVIDED

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

Centrifuge;

37 ℃ 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℃-25℃) before use.

2. As recommended in Table 2, the lyophilized materials of HRP-Conjugated Antibody 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℃, Do not thaw and freeze more than 3 times.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

Catalog	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
RAS045-C05	HRP-Conjugated Antibody	10 µg	50 µg/mL	200 µL water

RECOMMENDED SAMPLE PREPARATION

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

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 Positive Control and Negative Control working fluid and pre-treatment of samples:

a. For qualitative detection of antibodies:

Dilute the samples, Positive Control and Negative Control at 1:100 with Dilution Buffer.

b. For semi-quantitative detection or titer measurement of antibodies:

b.1 It is recommended dilute the samples from 1:100-1: 51200 with Dilution Buffer.

b.2 If titer measurement,it is recommended dilute the Positive Control and Negative Control from 1:100-1: 51200 with Dilution Buffer.

If semi-quantitative detection,it is recommended dilute the Anti-SARS-CoV-2 Antibody (Control, Mouse IgG) from 0.098-3.125 ng/mL with Dilution Buffer. Please refer to the tube label for concentration.

2. Plate set up

Number the diluted samples corresponding to the wells of the Pre-coated with SARS-CoV-2 Spike S1 Microplate.

Each experiment requires a set of Positive Control and Negative Control working fluid.

3. Add Samples

Add 100 µL diluted sample, Positive Control and Negative Control working fluid to the corresponding wells. Add 100 µL Dilution Buffer to blank control. Seal the plate with microplate sealing film and incubate at 37℃ for 1.0 h.

4. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, gently tap the plate for 1 min, 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.

5. Add HRP-Conjugated Antibody

Dilute HRP-Conjugated Antibody stock solution (50 µg/mL) to 0.08 µg/mL with Dilution Buffer to make a working solution. The prepared working fluid should be stored away from light. For all wells, add 100 µL HRP-Conjugated

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

Antibody working solution. Seal the plate with microplate sealing film and incubate at 37℃ for 1.0 h, avoid light.

6. Washing

Repeat step 4.

7. Substrate Reaction

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

8. 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.*

9. Data Recording

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

Note*: To reduce the background noise, substract the value read at* $OD_{450\ nm}$ *with the value read at* $OD_{630\ nm}$.

CUT-OFF VALUE IDENTIFICATION

Cut-off value =0.1

Normal range of Negative control (1:100): $OD_{450\ nm}$-$OD_{630\ nm}$＜0.1

Normal range of Positive control (1:3200): $OD_{450\ nm}$-$OD_{630\ nm}$≥1.2

Note：The cut-off value can be determined by the end user.

INTERPRETION OF RESULTS

Positive reading: $OD_{450\ nm}$-$OD_{630\ nm}$ of sample ≥ Cut-off value means Anti-SARS-CoV-2 Antibody IgG (Spike S1) are detected.

Negative reading: $OD_{450\ nm}$-$OD_{630\ nm}$ of sample ＜ Cut-off value means Anti-SARS-CoV-2 Antibody IgG (Spike S1) are not detected.

a. 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.

b. For semi-quantitative detection of antibodies:

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com

If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated. To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is subtracted to the OD value of the blank control. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Linear regression equation is used to draw the standard curve and calculate the sample concentration.

LIMITATIONS OF THE PROCEDURE

The kit cannot be used for quantitative detection.

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℃-25℃) 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, all prepared working solution is for one-time use and cannot be stored.

US and Canada: Tel: +1 800-810-0816
Asia and Pacific: Tel: +86 400-682-2521
Web: http://www.acrobiosystems.com
E-mail: order@acrobiosystems.com