T152-EN.01

Human Anti-SARS-CoV-2 (BF.7) Antibody IgA Titer Serologic Assay Kit (Spike RBD)

Pack Size: 96 tests

Catalog Number： RAS-T152

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 product is developed for titer measurement of Anti-SARS-CoV-2(BF.7) Antibody IgA (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 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 IgA by employing an indirect ELISA. Immobilize SARS-CoV-2 Spike RBD (BF.7) on the microplate. Then add the samples, incubate and wash the wells. Next add Secondary antibody HRP-Anti-Human IgA 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 color 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
RAS152-C01	Pre-coated SARS-CoV-2 Spike RBD (BF.7) Microplate	1 plate	Solid	2-8°C	2-8°C
RAS152-C02	Anti-SARS-CoV-2 Antibody (Control, IgA)	100 µL	Liquid	2-8°C	2-8°C
RAS152-C03	HRP-Anti-Human IgA	50 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS152-C04	10 x Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS152-C05	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS152-C06	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS152-C07	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

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STORAGE AND VALIDITY INSTRUCTIONS

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

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 ºC 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

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in a 37 ºC incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

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 Control, IgA working fluid and pre-treatment of samples:

a. It is recommended to dilute the samples from 1:100-1:25600 with Dilution Buffer.

b. It is recommended dilute the Anti-SARS-CoV-2 Antibody (Control, IgA) from 31.25-0.98 ng/mL with Dilution Buffer. Please refer to the tube label for concentration.

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2. Plate set up

Number the diluted samples corresponding to the wells of the Pre-coated SARS-CoV-2 Spike RBD (BF.7) Microplate. Each experiment requires a set of Control IgA working fluid.

3. Add Samples

Add 100 µL diluted samples and Control IgA 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-Anti-Human IgA

Dilute HRP-Goat anti-Human IgA at 1:3000 with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

For all wells, add 100 µL HRP-Anti-Human IgA 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, subtract the value read at* $OD_{450\ nm}$ *with the value read at* $OD_{630\ nm}$.

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CUT-OFF VALUE IDENTIFICATION

Cut-off value =0.1

Normal range of control IgA (15.6ng/mL): $OD_{450\ nm}$-$OD_{630\ nm}$≥1.0

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

INTERPRETION OF RESULTS

a. For qualitative detection of antibodies:

Positive reading: $OD_{450\ nm}$-$OD_{630\ nm}$ of sample ≥ Cut-off value means Anti-SARS-CoV-2(BF.7) Antibody IgA (Spike RBD) are detected.

Negative reading: $OD_{450\ nm}$-$OD_{630\ nm}$ of sample ＜ Cut-off value means Anti-SARS-CoV-2(BF.7) Antibody IgA (Spike RBD) are not detected.

b. 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.

c. For semi-quantitative detection of antibodies:

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. Four parameters logistic or other statistical software are 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.

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4. Bring all reagents and samples to room temperature (20℃-25℃) before use.
5. If crystals have formed in the buffer solution, incubate until the crystals have completely dissolved. Before use, bring the solution back to room temperature.
6. This kit should be stored at 2°C -8°C.
7. 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.

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