T151-EN.01

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

Pack Size: 96 tests

Catalog Number： RAS-T151

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 (BF.7) Antibody IgM (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 IgM 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 IgM 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
RAS151-C01	Pre-coated SARS-CoV-2 Spike RBD (BF.7) Microplate	1 plate	Solid	2-8°C	2-8°C
RAS151-C02	SARS-CoV-2 Antibody Positive Control	100 µL	Liquid	2-8°C	2-8°C
RAS151-C03	SARS-CoV-2 Antibody Negative Control	100 µL	Liquid	2-8°C	2-8°C
RAS151-C04	HRP-Anti-Human IgM	100 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS151-C05	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS151-C06	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS151-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light

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

RAS151-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

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;

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.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) 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 SARS-CoV-2 Antibody Positive Control and SARS-CoV-2 Antibody Negative Control working fluid and pre-treatment of samples:

a. For qualitative detection of antibodies:

Dilute the samples, Positive Control and Negative Control at 1:50 with Dilution Buffer.

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

b. For determination of antibody titer:

It is recommended to dilute the samples, SARS-CoV-2 Antibody Positive Control and SARS-CoV-2 Antibody Negative Control from 1:50-1:25600 with Dilution Buffer.

1.3 Preparation of **HRP-Anti-Human IgM** working fluid:

Dilute **HRP-Anti-Human IgM** at 1:1000 with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

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 SARS-CoV-2 Antibody Positive Control and SARS-CoV-2 Antibody Negative Control working fluid.

3. Add Samples

Add 100 µL diluted Samples, SARS-CoV-2 Antibody Positive Control and SARS-CoV-2 Antibody 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 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 IgM working fluid

Add 100 µL HRP-Anti-human IgM 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 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at 37℃ for 20 min. Avoid light.

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

8. Termination

Add 50 μL **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 450nm and 630nm using UV/Vis microplate spectrophotometer.

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

CUT-OFF VALUE IDENTIFICATION

Cut-off value =0.100

Normal range of Negative control (1:50): OD450 nm-OD630 nm＜0.100

Normal range of Positive control (1:400): OD450 nm-OD630 nm≥1.500

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 IgM (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 IgM (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.

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.

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

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℃-8℃.
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.

TYPICAL DATA

Note: *The Typical data is for reference only.*

a. For qualitative detection of antibodies:

Value Result in units	Result	Test Result Interpretation
$OD_{450\ nm}$ - $OD_{630\ nm}$ =0.031	Negative	Anti-SARS-CoV-2(BF.7) Antibody IgM (Spike RBD) are not detected
$OD_{450\ nm}$ - $OD_{630\ nm}$ =0.247	Positive	Anti-SARS-CoV-2(BF.7) Antibody IgM (Spike RBD) are detected

b. For determination of antibody titer:

Note: *Quality control data between different plates should not be mixed, and negative and positive controls should be set for each test.*

Ratio of Dilution	OD450nm-OD630nm (Samples)	Result
50	3.008	The titer level of antibody is 12800
100	3.024	
200	2.850	
400	2.458	
800	1.662	
1600	0.996	
3200	0.569	
6400	0.364	
12800	**0.189**	
25600	0.094	
Blank	0.018	

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