T114-EN.01

Mouse Anti-SARS-CoV-2 (BA.2.12.1) Antibody IgG Titer Serologic Assay Kit (Spike Trimer)

Pack Size: 96 tests

Catalog Number： RAS-T114

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

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

INTENDED USE

The kit is developed for titer measurement of Anti-SARS-CoV-2(BA.2.12.1) Antibody IgG (Spike Trimer) in mouse 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 IgG by employing an indirect ELISA. Immobilize SARS-CoV-2 Spike Trimer (BA.2.12.1) on the microplate. Then add the samples, incubate and wash the wells. Next add Secondary antibody HRP-Anti-Mouse 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
RAS114-C01	Pre-coated SARS-CoV-2 Spike Trimer (BA.2.12.1) Microplate	1 plate	Solid	2-8°C	2-8°C
RAS114-C02	Positive Control	100 µL	Liquid	2-8°C	2-8°C
RAS114-C03	Negative Control	100 µL	Liquid	2-8°C	2-8°C
RAS114-C04	HRP-Goat anti-Mouse IgG	50 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS114-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS114-C06	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS114-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS114-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

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E-mail: order@acrobiosystems.com

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

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.

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 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.

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b. For determination of antibody titer:

It is recommended to dilute the samples, Positive Control and Negative Control from 1:100-1:25600 with Dilution Buffer.

2. Plate set up

Number the diluted samples corresponding to the wells of the Pre-coated SARS-CoV-2 Spike Trimer (BA.2.12.1) Microplate. Each experiment requires a set of Positive Control and Negative Control working fluid.

3. Add Samples

Add 100 μL diluted samples, 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-Goat anti-Mouse IgG

Dilute HRP-Goat anti-Mouse IgG solution at 1:1000 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-Goat anti-Mouse IgG 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.

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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* $OD_{630\ nm}$.

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 (1:800): $OD_{450\ nm}$-$OD_{630\ nm}$≥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(BA.2.12.1) Antibody IgG (Spike Trimer) are detected.

Negative reading: $OD_{450\ nm}$-$OD_{630\ nm}$ of sample ＜ Cut-off value means Anti-SARS-CoV-2(BA.2.12.1) Antibody IgG (Spike Trimer) 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.
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

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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 1xWashing 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
$OD_{450\ nm}$ - $OD_{630\ nm}$ =0.042	Negative	Anti-SARS-CoV-2(BA.2.12.1) Antibody IgG (Spike Trimer) are not detected
$OD_{450\ nm}$ - $OD_{630\ nm}$ =0.319	Positive	Anti-SARS-CoV-2(BA.2.12.1) Antibody IgG (Spike Trimer) 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	$OD_{450\ nm}$ - $OD_{630\ nm}$ (**Samples**)	Result
100	3.255	The titer level of antibody is 25600
200	3.437	
400	3.226	
800	2.581	
1600	1.732	
3200	0.983	
6400	0.516	
12800	0.285	
25600	**0.129**	
51200	0.059	
Blank	0.022	

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