A021-EN.02

SARS-CoV-2 Spike RBD Titer Assay Kit

Pack Size: 96tests

Catalog Number： RAS-A021

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 kit is developed for detecting SARS-CoV-2 Spike RBD in the sample.

It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

The newly identified Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is posing a serious threat to human health. A rapid and effective assay kit detecting the levels of SARS-CoV-2 Spike RBD is urgently needed to accelerate the development of COVID-19 vaccines.

This assay kit is used to measure the levels of SARS-CoV-2 Spike RBD by employing a standard sandwich-ELISA format. The microplate in the kit has been pre-coated with Anti-SARS-CoV-2 Spike RBD Antibody. First add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-SARS-CoV-2 Spike RBD Antibody to the plate, incubate and wash the wells. Next add Streptavidin-HRP 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 protein present. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm/630 nm. The OD Value reflects the amount of protein bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

ID	Components	Size (96tests)	Format	Storage	
				Unopened	Opened
RAS021-C01	Pre-coated with Anti-SARS-CoV-2 Spike RBD Antibody Microplate	1 plate	Solid	2-8℃	2-8℃
RAS021-C02	SARS-CoV-2 Spike RBD	10 μg	Powder	2-8℃	-70℃
RAS021-C03	Biotin-Anti-SARS-CoV-2 Spike RBD Antibody	10 μg	Liquid	2-8℃	2-8℃
RAS021-C04	Streptavidin-HRP	10 μg	Powder	-2-8℃, avoid light	-70℃, avoid light
RAS021-C05	10×Washing Buffer	50 mL	Liquid	2-8℃	2-8℃
RAS021-C06	Dilution Buffer	50 mL	Liquid	2-8℃	2-8℃
RAS021-C07	Substrate Solution	12 mL	Liquid	-2-8℃, avoid light	-2-8℃, avoid light
RAS021-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

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

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or dual wavelength microplate reader with 450 nm /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;

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.

RECONSTITUTION

Reconstitute the provided lyophilized materials to stock solutions with distilled, sterile water as recommended in **Table 2** and place the materials for 15 to 30 minutes at room temperature with occasional gentle mixing. **Avoid vigorous shaking**. 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 5µg per vial.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

ID	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
RAS021-C02	SARS-CoV-2 Spike RBD	10 µg	50 µg/mL	200 µL water
RAS021-C04	Streptavidin-HRP	10 µg	50 µg/mL	200 µL water

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

REAGENT PREPARATION

Bring all reagents and samples to preparation temperature (20℃-25℃) before use.If crystals are observed in buffer solution, place the reagents at room temperature until the crystals completely dissolved.

1×Washing Buffer: prepare 500 mL 1× Washing Buffer by adding 50 mL of 10 × Washing Buffer to 450mL distilled water.

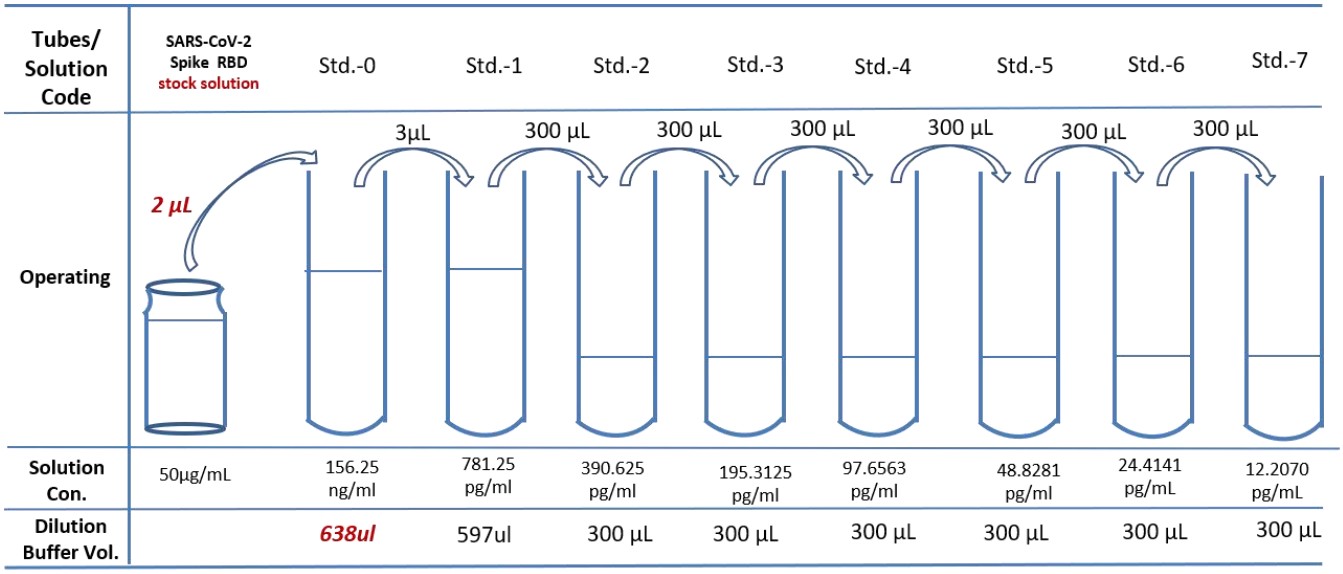
Reconstitute and store all reagents as recommended in Table2.

RECOMMENDED PROTOCOL

1. Preparation of Standard curve

Make serial dilutions of the **SARS-CoV-2 Spike RBD** as a Standard curve with **Dilution Buffer** as recommended in **Figure 1**.

FIGURE 1. PREPARATION OF 1:2 SERIAL DILUTIONS OF THE SARS-CoV-2 Spike RBD



2. Add Samples

Add 100 µL serially diluted SARS-CoV-2 Spike RBD Standard curve and samples to each well. For blank control wells, please add 100 µL Dilution Buffer. Seal the plate with microplate sealing film and incubate at 37℃ for 1.0 h. Avoid light.

3. Washing

Remove the remaining solution by aspiration, add 300 µL **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

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

towels. Repeat the wash step above for three times.

4. Biotin-Anti-SARS-CoV-2 Spike RBD Antibody

1) Dilute **Biotin-Anti-SARS-CoV-2 Spike RBD Antibody** solution (100 μg/mL) to 0.5 μg/mL with Dilution Buffer to make Biotin-Anti-SARS-CoV-2 Spike RBD Antibody working solution.
2) Add 100 μL **Biotin-Anti-SARS-CoV-2 Spike RBD Antibody** working solution to all wells, seal the plate with microplate sealing film and incubate at 37℃ for 1.0 h. Avoid light.

5. Washing

Repeat step 3.

6. Streptavidin-HRP

1) Dilute **Streptavidin-HRP** stock solution(50 μg/mL) to 0.1 μg/mL with Dilution Buffer to make Streptavidin-HRP working solution.
2) Add 100 μL **Streptavidin-HRP** working solution, seal the plate with microplate sealing film and incubate at 37℃ for 1.0 h. Avoid light.

7. Washing

Repeat step 3.

8. 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.

9. 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.*

10. Data Recording

Read the absorbance at 450 nm/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* $OD_{630\,nm}$.

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

CALCULATION OF RESULTS

1. Quality standards of Linearity： correlation coefficient R^2>0.9900。

2. Normal range of Std.-1(781.25 pg/mL): OD Value＞1.0

Note：If* OD *values or Linearity of controls do not meet the requirement, the test is invalid and must be repeated.

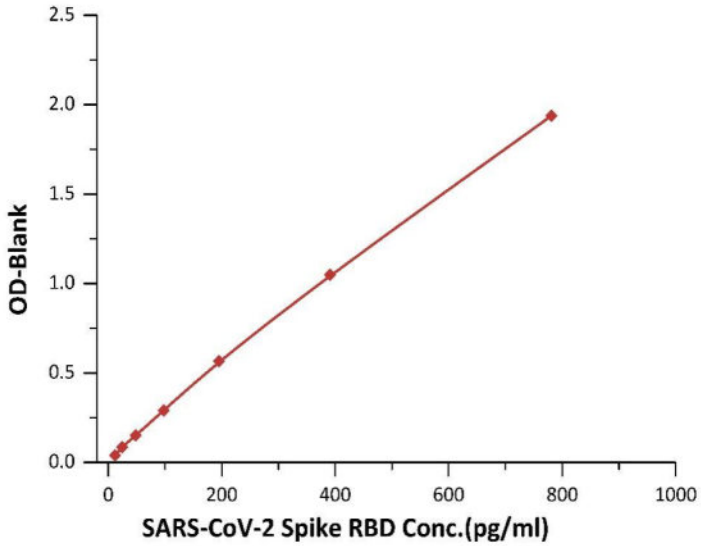
3. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.

4. To calibrated 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.

TYPICAL DATA

The following data are for reference only, and the sample concentration is calculated according to the tested standard curve.

	SARS-Cov-2 Spike RBD (pg/mL)	OD-Blank
Std.-1	781.25	1.9386
Std.-2	390.625	1.0496
Std.-3	195.3125	0.5676
Std.-4	97.6563	0.2906
Std.-5	48.8281	0.1516
Std.-6	24.4141	0.0866
Std.-7	12.2070	0.0396



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