A118-EN.01

SARS-CoV-2 Spike Trimer (BA.4) ELISA Kit

Pack Size: 96 tests

Catalog Number：RAS-A118

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 Trimer (BA.4) 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 Trimer 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 Trimer (BA.4) by employing a standard sandwich-ELISA format. The microplate in the kit has been pre-coated with Anti-SARS-CoV-2 Spike Trimer Antibody. First add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Next add Secondary antibody HRP-Anti-SARS-CoV-2 Spike Trimer 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 Spike Trimer (BA.4) 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 Spike Trimer bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS118-C01	Pre-coated Anti-SARS-CoV-2 Spike Trimer Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RAS118-C02	SARS-CoV-2 Spike Trimer (BA.4)	10 μg	Powder	2-8°C	-70°C
RAS118-C03	HRP-Anti-SARS-CoV-2 Spike Trimer Antibody	30 μg	Powder	2-8°C, avoid light	-70°C, avoid light
RAS118-C04	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS118-C05	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS118-C06	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS118-C07	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

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REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

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

Centrifuge;

37ºC Incubator;

10 µL, 200 µL and 1000 µL precision pipettes;

10 µL, 200 µL and 1000 µL pipette tips;

Multichannel pipettes;

Tubes;

Graduated cylinder to prepare Wash Solution;

Deionized or distilled water to dilute 10× Washing Buffer;

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.

REAGENT PREPARATION

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

2. 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℃. It is recommended not to freeze-thaw more than 2 times, the packing specification shall not be less than 5µg.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

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ID	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
RAS118-C02	SARS-CoV-2 Spike Trimer (BA.4)	10 μg	100 μg/mL	100 μL water
RAS118-C03	HRP-Anti-SARS-CoV-2 Spike Trimer Antibody	30 μg	100 μg/mL	300 μL water

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.

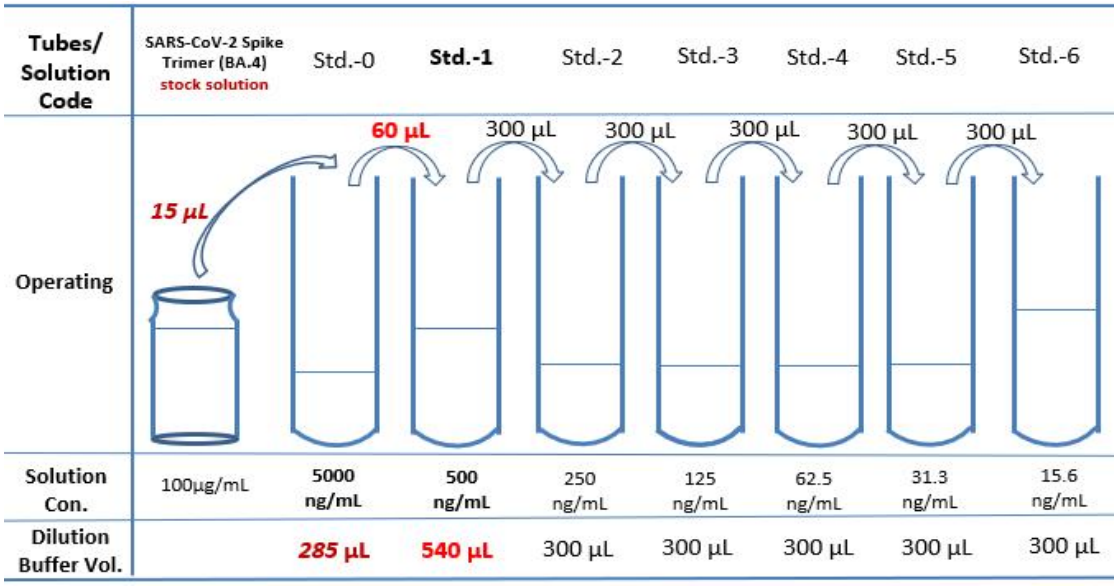
1.2 Preparation of HRP-Anti-SARS-CoV-2 Spike Trimer Antibody working fluid:

Dilute HRP-Anti-SARS-CoV-2 Spike Trimer Antibody to 2.0 μg/mL with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

2. Preparation of Standard curve

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

FIGURE 1. PREPARATION OF 1:1 SERIAL DILUTIONS OF THE SARS-CoV-2 Spike Trimer (BA.4)



3. Add Samples

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Add 100μL serially diluted SARS-CoV-2 Spike Trimer (BA.4) Standard curve and samples to each well. For blank Control wells, please add 100μL Dilution Buffer. Seal the plate with microplate sealing film and incubate at 37℃ for 1 hour.

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.Abb HRP-Anti-SARS-CoV-2 Spike Trimer Antibody

For all wells, add 100 μL HRP-Anti-SARS-CoV-2 Spike Trimer Antibody (dilute to 2.0 μg/mL) working solution. Seal the plate with microplate sealing film and incubate at 37℃ for 1 hour, 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* $OD_{630\ nm}$.

CALCULATION OF RESULTS

1. Normal range of Standard curve: R2≥0.9900, detection range: 15.6-500 ng/mL .

2. 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.

3. To calibrate absorbance value obtained by the standard curve, the OD value of the sample to be measured is

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subtracted from 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 or Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

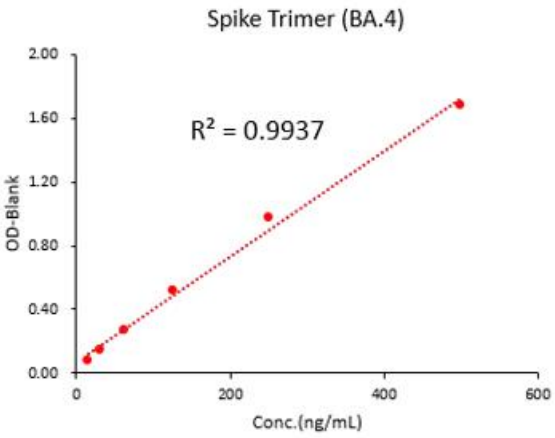
PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic procedures.
2. The kit should be used according to the instructions.
3. Do not mix reagents from different lots.
4. All reagents should be balance to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, warm to room temperature until the crystals have completely dissolved.
5. The kit should be stored at 2°C to 8°C.

TYPICAL DATA

The following data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Spike Trimer (BA.4) (ng/mL)	OD450-630nm	OD450-630nm-Blank
500	1.734	1.681
250	1.027	0.974
125	0.574	0.521
62.5	0.323	0.270
31.25	0.195	0.142
15.625	0.133	0.080
Blank	0.053	0.000



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