A170-EN.01

SARS-CoV-2 Spike S1 (B.1.1.529) Specific ELISA Kit
(For Vaccine Development)

Pack Size: 96 tests

Catalog Number：RAS-A170

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 kit is developed for detecting SARS-CoV-2 Spike S1 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) has posed a serious threat to human health. A rapid and effective assay kit detecting the levels of SARS-CoV-2 Spike S1 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 S1 (B.1.1.529) by employing a standard sandwich-ELISA format. The microplate in the kit has been pre-coated with Anti-SARS-CoV-2 Spike S1 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 S1 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 S1 (B.1.1.529) 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 S1 bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS170-C01	Pre-coated Anti-SARS-CoV-2 Spike S1 (B.1.1.529) Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RAS170-C02	SARS-CoV-2 Spike S1 (B.1.1.529) Standard	30 μg	Powder	2-8°C	-70°C
RAS170-C03	HRP-Anti-SARS-CoV-2 Spike S1 Antibody	20 μg	Powder	2-8°C	-70°C
RAS170-C04	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS170-C05	2xDilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS170-C06	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS170-C07	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

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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;

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.

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 a 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 1 times, the packing specification shall not be less than 5 µg.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

ID	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
RAS170-C02	SARS-CoV-2 Spike S1 (B.1.1.529) Standard	30 µg	200 µg/mL	150 µL water
RAS170-C03	HRP-Anti-SARS-CoV-2 Spike S1 Antibody	20 µg	200 µg/mL	100 µL water

RECOMMENDED SAMPLE PREPARATION

1. Working fluid preparation

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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 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

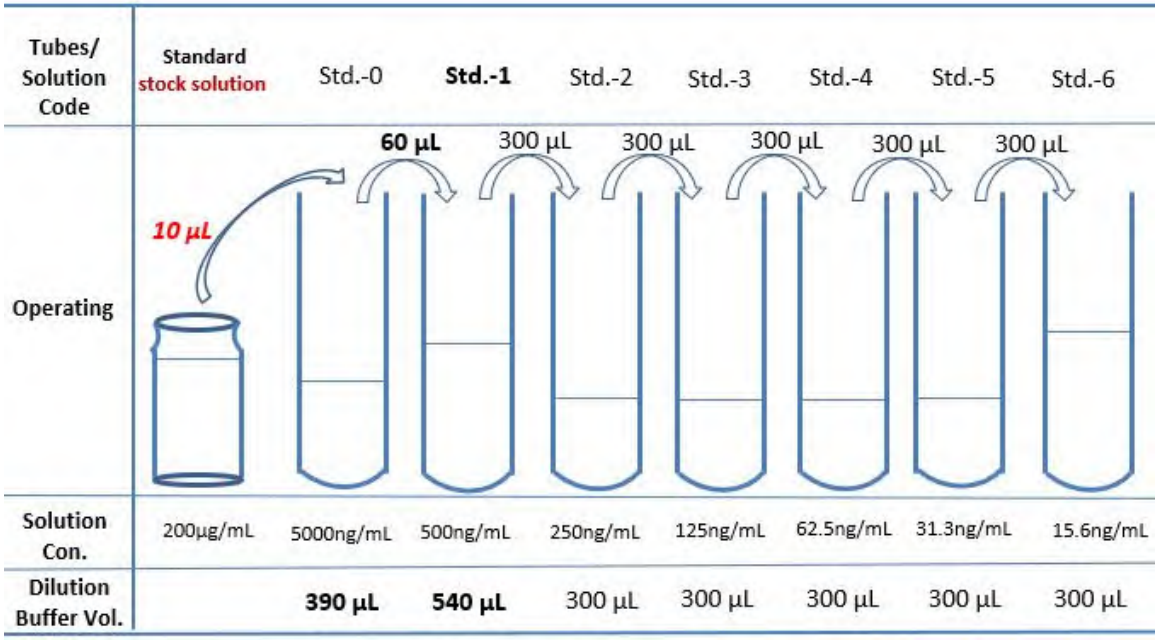
1.3 Preparation of HRP-Anti-SARS-CoV-2 Spike S1 Antibody working fluid:

Dilute HRP-Anti-SARS-CoV-2 Spike S1 Antibody to 0.5 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 S1 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 S1



3. Add Samples

Add 100 μL serially diluted SARS-CoV-2 Spike S1 Standard curve and samples to each well. For blank Control wells, please add 100 μL 1×Dilution Buffer. Seal the plate with microplate sealing film and incubate at 37°C for 1 hour.

Note: *It is recommended to set multiple holes for samples and standard curves to be measured.*

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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-SARS-CoV-2 Spike S1 Antibody

For all wells, add 100 µL **HRP-Anti-SARS-CoV-2 Spike S1 Antibody (dilute to 0.5 µg/mL)** working solution. Seal the plate with microplate sealing film and incubate at 37℃ for 1 hour.

6. Washing

Repeat step 4.

9. 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.

10. Termination

Add 50 µL **Stop Solution** to each well, and tap the plate gently for 5 min to allow thorough mixing.

Note: *the color in the wells should change from blue to yellow.*

11. 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: $R^2 \geq 0.9900$, 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 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 are used to draw the standard curve and calculate the sample concentration.

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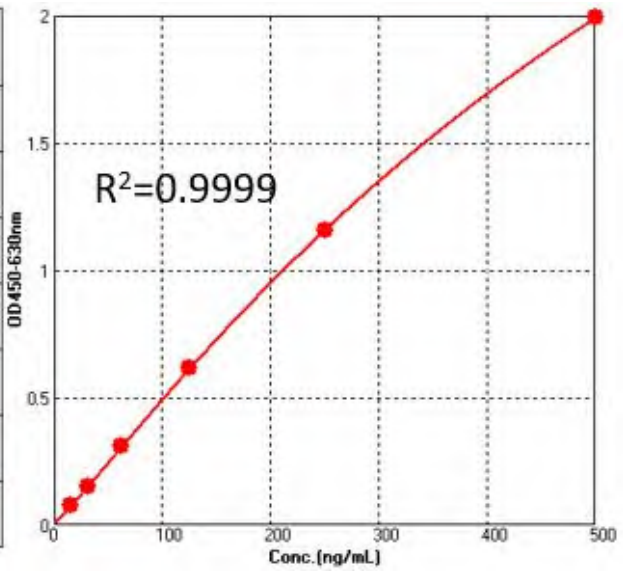
PRECAUTIONS

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. Bring all reagents and samples 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

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only.

SARS-CoV-2 Spike S1 Standard(ng/mL)	OD450-630nm
500	1.991
250	1.159
125	0.616
62.5	0.310
31.25	0.150
15.625	0.079
0	0.015



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