A145-EN.01

SARS-CoV-2 Spike Trimer (XBB.1.5) ELISA Kit (For Vaccine Development)

Pack Size: 96 tests

Catalog Number： RAS-A145

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 (XBB.1.5) in the sample, simultaneously can detect Omicron (BA.2 and BA.4 and BA.5 and BQ.1.1).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 (XBB.1.5) 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. Then add the Biotin-Anti-SARS-CoV-2 Spike Trimer 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 Spike Trimer (XBB.1.5) 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
RAS145-C01	Pre-coated Anti-SARS-CoV-2 Spike Trimer Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RAS145-C02	SARS-CoV-2 Spike Trimer (XBB.1.5)	15 μg	Powder	2-8°C	-70°C
RAS145-C03	Biotin-Anti-SARS-CoV-2 Spike Trimer Antibody	100 μL	Liquid	2-8°C	2-8°C
RAS145-C04	Streptavidin-HRP	10 μg	Powder	2-8°C, avoid light	-70°C, avoid light
RAS145-C05	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS145-C06	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C

RAS145-C07	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
RAS145-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

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

Centrifuge;

37° C 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 AND EXPIRATION DATE

The unopened kit is stable for 12 months from the date of manufacture if stored at 2°C to 8°C.

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 37ºC 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.
RAS145-C02	SARS-CoV-2 Spike Trimer (XBB.1.5)	15 μg	100 ug/mL	150 μL water
RAS145-C04	Streptavidin-HRP	10 μg	100 ug/mL	100 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 Biotin-Anti-SARS-CoV-2 Spike Trimer Antibody working fluid:

Dilute Biotin-Anti-SARS-CoV-2 Spike Trimer Antibody at 1:500 with Dilution Buffer. Please prepare it for one-time use only.

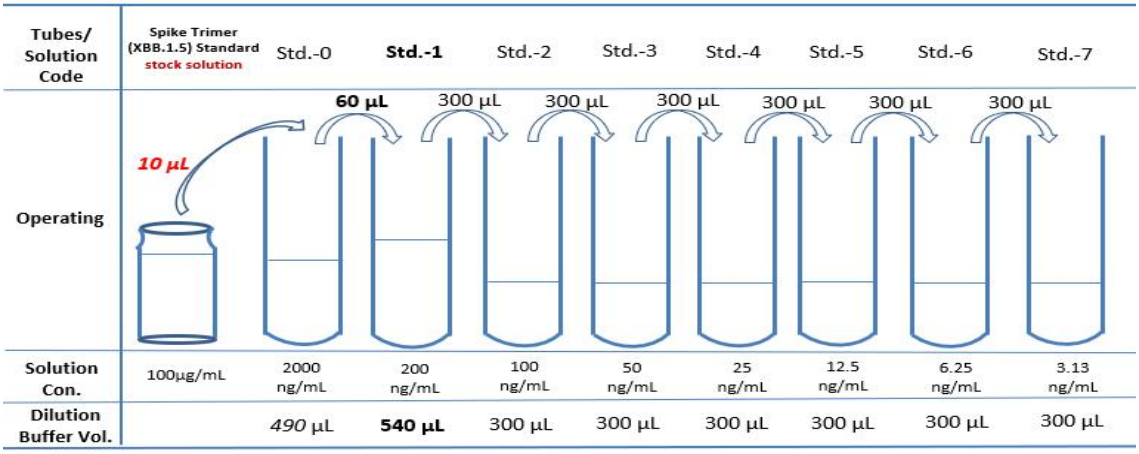
1.3 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP to 0.05 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 (XBB.1.5)



3. Add Samples

Add 100 μL serially diluted SARS-CoV-2 Spike Trimer (XBB.1.5) 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. Add Biotin-Anti-SARS-CoV-2 Spike Trimer Antibody

For all wells, add 100 μL **Biotin -Anti-SARS-CoV-2 Spike Trimer Antibody (dilute at 1:500)** working solution. Seal the plate with microplate sealing film and incubate at 37℃ for 1 hour, avoid light.

6. Washing

Repeat step 4.

7. Add Streptavidin-HRP

For all wells, add 100 μL **Streptavidin-HRP (dilute to 0.05 μg/mL)** working solution. Seal the plate with microplate sealing film and incubate at 37℃ for 1 hour, avoid light.

8. 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 3 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: 200-3.13 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 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 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 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 10xWashing Buffer, all prepared working solution is for one-time use and cannot be stored.

TYPICAL DATA

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

Spike Trimer (XBB.1.5) Standard(ng/mL)	OD450-630nm	OD450-630nm-Blank
200	1.935	1.901
100	1.094	1.060
50	0.583	0.549
25	0.333	0.299
12.5	0.180	0.146
6.25	0.101	0.067
3.125	0.067	0.033
Blank	0.034	0.000