

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

Pack Size: 96 tests

Catalog Number: RAS-A194

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 quantitative detection of SARS-CoV-2 Spike RBD (XBB.1.5) in samples. It is intended for research use only (RUO).

BACKGROUND

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 RBD is urgently needed to accelerate the development of COVID-19 vaccines.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of SARS-CoV-2 Spike RBD (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 RBD (XBB.1.5) 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 SARS-CoV-2 Spike RBD (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 SARS-CoV-2 Spike RBD (XBB.1.5) bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

| Catalog | Components | Size (96 tests) | Format | Storage | |
|------------|---|--------------------|--------|--------------------|--------------------|
| | | | | Unopened | Opened |
| RAS194-C01 | Pre-coated Anti-SARS-CoV-2 Spike RBD (XBB.1.5) Antibody Microplate | 1 plate | Solid | 2-8°C | 2-8°C |
| RAS194-C02 | SARS-CoV-2 Spike RBD (XBB.1.5) | 15 µg | Powder | 2-8°C | -70°C |
| RAS194-C03 | Biotin-Anti-SARS-CoV-2 Spike RBD Antibody | 100 µL | Liquid | 2-8°C | 2-8°C |
| RAS194-C04 | Streptavidin-HRP | 50 µL | Liquid | 2-8°C, avoid light | 2-8°C, avoid light |

| | | | | | |
|------------|--------------------|-------|--------|--------------------|--------------------|
| RAS194-C05 | 10×Washing Buffer | 50 mL | Liquid | 2-8°C | 2-8°C |
| RAS194-C06 | Dilution Buffer | 50 mL | Liquid | 2-8°C | 2-8°C |
| RAS194-C07 | Substrate Solution | 12 mL | Liquid | 2-8°C, avoid light | 2-8°C, avoid light |
| RAS194-C08 | Stop Solution | 7 mL | Liquid | 2-8°C | 2-8°C |

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

1. Unopened kit should be stored at 2°C-8°C upon receiving.
2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.
3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, place the sample in a 37 °C 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°C. 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. |
|------------|--------------------------------|-------|---------------------|--------------------------------|
| RAS194-C02 | SARS-CoV-2 Spike RBD (XBB.1.5) | 15 µg | 100 µg/mL | 150 µL water |

RECOMMENDED SAMPLE PREPARATION

1. Working fluid preparation

1.1 Preparation of 1×Washing Buffer: n

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of Biotin-Anti-SARS-CoV-2 Spike RBD Antibody working fluid:

Dilute Biotin-Anti-SARS-CoV-2 Spike RBD Antibody to 0.4 µg/mL with Dilution Buffer. Please prepare it for one-time use only.

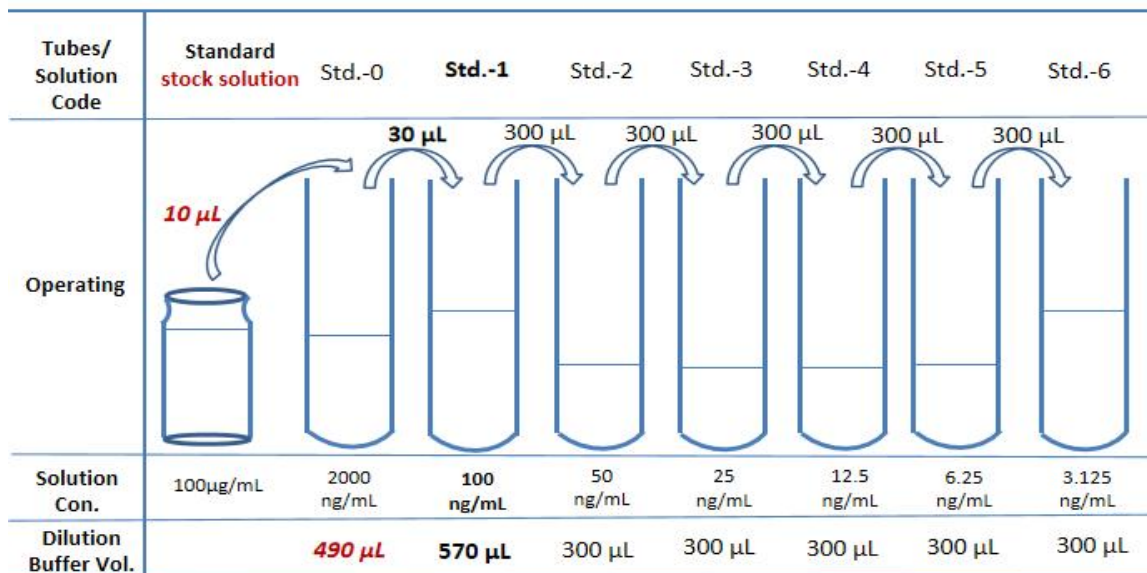
1.3 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:2000 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 RBD (XBB.1.5) 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 RBD (XBB.1.5)



3. Add Samples

Add 100 μL serially diluted SARS-CoV-2 Spike RBD (XBB.1.5) 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 room temperature for 1 hour.

4. Washing

Remove the remaining solution by aspiration, add 300 μL of 1 \times Washing Buffer to each well, gently tap the plate for 1 min, remove any remaining 1 \times 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 RBD Antibody

For all wells, add 100 μL **Biotin -Anti-SARS-CoV-2 Spike RBD Antibody (dilute to 0.4 $\mu\text{g}/\text{mL}$)** working solution. Seal the plate with microplate sealing film and incubate at room temperature for 1 hour, avoid light.

6. Washing

Repeat step 4.

7. Add Streptavidin-HRP

For all wells, add 100 μL **Streptavidin-HRP (dilute to 1:2000)** working solution. Seal the plate with microplate sealing film and incubate at room temperature 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 room temperature 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_{450nm} with the value read at OD_{630nm}.

CALCULATION OF RESULTS

1. Normal range of Standard curve: $R^2 \geq 0.9900$, detection range: 3.125-100 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 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°C-25°C) 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 RBD (XBB.1.5) Standard(ng/mL) | OD450-630nm | OD450-630nm-Blank |
|-------------------------------------|-------------|-------------------|
| 100 | 2.516 | 2.472 |
| 50 | 1.440 | 1.396 |
| 25 | 0.768 | 0.724 |
| 12.5 | 0.371 | 0.327 |
| 6.25 | 0.208 | 0.164 |
| 3.125 | 0.120 | 0.076 |
| Blank | 0.044 | 0.000 |

