



A196-EN.01

## **SARS-CoV-2 Spike RBD (BA.2.12.1) & Spike S1 (B.1.1.529) Specific ELISA Kit**

**Pack Size: 96 tests**

**Catalog Number: RAS-A196**

**IMPORTANT: Please carefully read this manual before performing your experiment.**

***For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures***

**[HTTP://WWW.ACROBIOSYSTEMS.COM](http://www.acrobiosystems.com)**

---

**INTENDED USE**

This kit is developed for specific detection of SARS-CoV-2 Spike RBD (BA.2.12.1) & Spike S1 (B.1.1.529) in vaccine samples, which can meet the needs of vaccine developers to establish antigen quantification methods for preclinical evaluation, vaccine production and quality control, and realize accurate quantification of vaccine antigen contents for COVID-19 vaccines of Omicron-specific boosters. 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 (BA.2.12.1) & 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 RBD(BA.2.12.1) & Spike S1(B.1.1.529) Antibody. First add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the HRP-Anti-SARS-CoV-2 Spike protein 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 SARS-CoV-2 Spike RBD (BA.2.12.1) & SARS-CoV-2 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 SARS-CoV-2 Spike RBD (BA.2.12.1) & SARS-CoV-2 Spike S1 (B.1.1.529) bound.

**MATERIALS PROVIDED**

**TABLE 1. MATERIALS PROVIDED**

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS196-C01	Pre-coated Anti-SARS-CoV-2 Spike RBD(BA.2.12.1) & Spike S1(B.1.1.529) Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RAS196-C02	SARS-CoV-2 Spike RBD (BA.2.12.1)	15 µg	Powder	2-8°C	-70°C

RAS196-C03	SARS-CoV-2 Spike S1 (B.1.1.529)	30 µg	Powder	2-8°C	-70°C
RAS196-C04	HRP-Anti-SARS-CoV-2 Spike protein Antibody	20 µg	Powder	2-8°C, avoid light	-70°C, avoid light
RAS196-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS196-C06	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS196-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS196-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. The unopened kit should be stored at 2°C-8°C upon receiving.
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°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.
RAS196-C02	SARS-CoV-2 Spike RBD (BA.2.12.1)	15 µg	150 µg/mL	100 µL water
RAS196-C03	SARS-CoV-2 Spike S1 (B.1.1.529)	30 µg	150 µg/mL	200 µL water
RAS196-C04	HRP-Anti-SARS-CoV-2 Spike protein Antibody	20 µg	200 µg/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 1×Dilution Buffer:

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

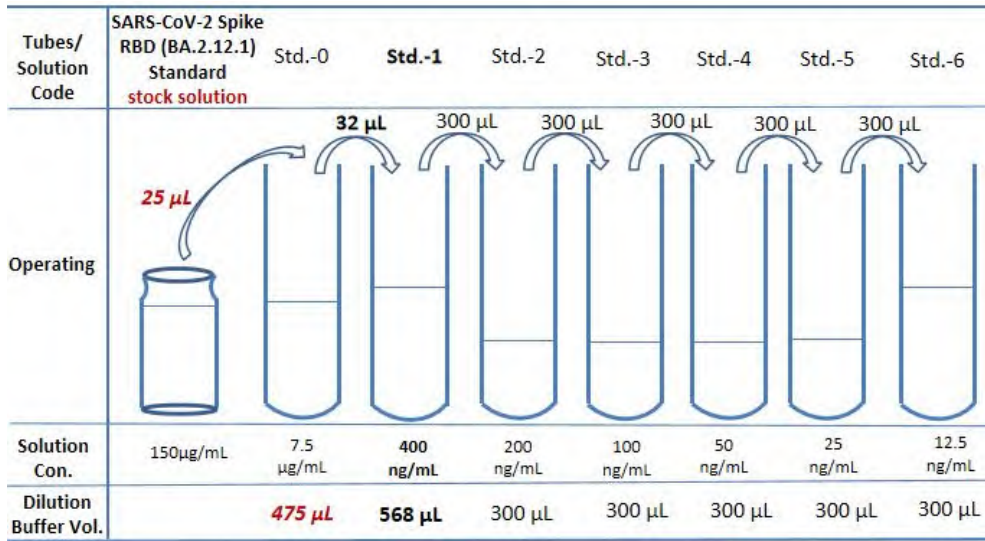
1.3 Preparation of HRP-Anti-SARS-CoV-2 Spike protein Antibody working fluid:

Dilute HRP-Anti-SARS-CoV-2 Spike protein Antibody to 1.0 µg/mL with Dilution Buffer. Please prepare it for one-time use only.

**2. Preparation of Standard curve**

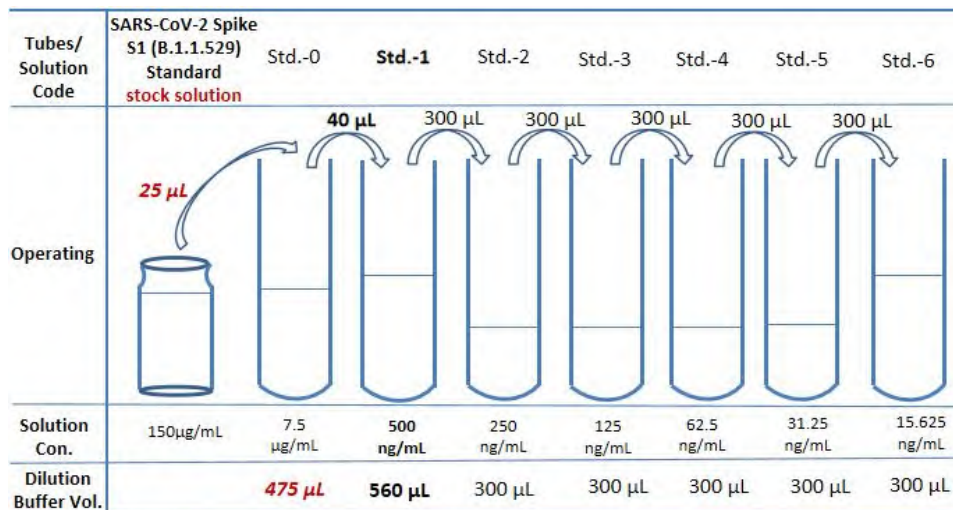
2.1 Make serial dilutions of the SARS-CoV-2 Spike RBD (BA.2.12.1) as a Standard curve with Dilution Buffer as recommended in Figure 1.

**FIGURE 1. PREPARATION OF 1:1 SERIAL DILUTIONS OF THE SARS-CoV-2 Spike RBD (BA.2.12.1)**



2.2 Make serial dilutions of the SARS-CoV-2 Spike S1 (B.1.1.529) as a Standard curve with Dilution Buffer as recommended in Figure 2.

FIGURE 2. PREPARATION OF 1:1 SERIAL DILUTIONS OF THE SARS-CoV-2 Spike S1 (B.1.1.529)



### 3. Samples

Add 100 µL serially diluted SARS-CoV-2 Spike RBD (BA.2.12.1) & SARS-CoV-2 Spike S1 (B.1.1.529) 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°C for 1.0 hour.

### 4. Washing

Remove the remaining solution by aspiration, add 300  $\mu$ L 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 HRP-Anti-SARS-CoV-2 Spike protein Antibody

For all wells, add 100  $\mu$ L **HRP-Anti-SARS-CoV-2 Spike protein Antibody (dilute to 1.0  $\mu$ g/mL)** working solution. Seal the plate with microplate sealing film and incubate at 37°C for 1.0 hour, avoid light.

## 6. Washing

Repeat step 4.

## 7. Substrate Reaction

Add 100  $\mu$ L **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at 37°C for 20 min, avoid light.

## 8. Termination

Add 50  $\mu$ L **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.*

## 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  $OD_{450\text{ nm}}$  with the value read at  $OD_{630\text{ nm}}$ .*

## CALCULATION OF RESULTS

1. Normal range of Standard curve:  $R^2 \geq 0.9900$ , SARS-CoV-2 Spike RBD (BA.2.12.1) detection range: 12.5-400 ng/mL, SARS-CoV-2 Spike S1 (B.1.1.529) detection range: 15.625-500 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. Linear regression equation or Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

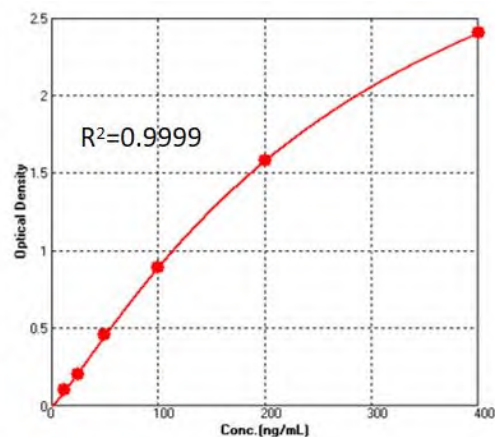
## PRECAUTIONS

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 (BA.2.12.1) Standard (ng/mL)	O.D.-1	O.D.-2	Average	Corrected
400	2.461	2.441	2.451	2.403
200	1.614	1.641	1.628	1.579
100	0.918	0.964	0.941	0.893
50	0.494	0.512	0.503	0.455
25	0.243	0.261	0.252	0.204
12.5	0.148	0.149	0.149	0.100
0	0.048	0.049	0.049	/



Spike S1 (B.1.1.529) Standard (ng/mL)	O.D.-1	O.D.-2	Average	Corrected
500	2.327	2.223	2.275	2.236
250	1.499	1.447	1.473	1.434
125	0.876	0.866	0.871	0.832
62.5	0.483	0.45	0.467	0.427
31.25	0.264	0.261	0.263	0.223
15.625	0.145	0.152	0.149	0.109
0	0.037	0.042	0.040	/

