N044-EN.01

Anti-SARS-CoV-2 Neutralizing Antibody Quantitative Detection Kit (Spike RBD)

Pack Size: 96 tests

Catalog Number： RAS-N044

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

For Research Use Only. Not for Use in Diagnostic and Therapeutic Applications

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INTENDED USE

This kit is developed for quantitative detection of Anti-SARS-CoV-2 neutralizing antibody (Spike RBD) in human serum. It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

This kit is developed for detecting neutralizing antibody against SARS-CoV-2 Spike RBD mutation in the sample through a competitive ELISA. The microplate in the kit is pre-coated with Human ACE2 protein. To initiate the experiment, samples and Calibrators are added to the wells followed by addition of HRP-SARS-CoV-2 Spike RBD. After incubation, the wells are washed and Substrate Solution is added to the wells. The reaction is terminated by the addition of Stop Solution and the intensity of absorbance is measured at 450 nm、630 nm. The neutralizing antibodies in the samples will compete with ACE2 for HRP-SARS-CoV-2 Spike RBD binding. The intensity of assay signal decrease proportionally with the concentration of Anti-SARS-CoV-2 neutralizing antibodies.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Amount (96 tests)	Format	Storage	
				Unopened	Opened
RAS044-C01	Pre-coated Human ACE2 Microplate	1 plate	Solid	2-8℃	2-8℃
RAS044-C02	Calibrator1	0.4 mL	Powder	2-8℃	-70℃
RAS044-C03	Calibrator2	0.4 mL	Powder	2-8℃	-70℃
RAS044-C04	Calibrator3	0.4 mL	Powder	2-8℃	-70℃
RAS044-C05	Calibrator4	0.4 mL	Powder	2-8℃	-70℃
RAS044-C06	Calibrator5	0.4 mL	Powder	2-8℃	-70℃
RAS044-C07	Calibrator6	0.4 mL	Powder	2-8℃	-70℃
RAS044-C08	HRP-SARS-CoV-2 Spike RBD	15 μg	Powder	2-8℃, avoid light	-70℃, avoid light
RAS044-C09	10xWashing Buffer	50 mL	Liquid	2-8℃	2-8℃
RAS044-C10	Dilution Buffer	50 mL	Liquid	2-8℃	2-8℃
RAS044-C11	Substrate Solution	12 mL	Liquid	2-8℃, avoid light	2-8℃, avoid light
RAS044-C12	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

STORAGE

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.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or dual wavelength microplate reader with 450 nm、630 nm；

37 ºC Incubator；

Single channel or multichannel pipettes with 10 µL, 200 µL and 1000 µL precision；

10 µL, 200 µL and 1000 µL pipette tips；

Test Tubes；

Graduated cylinder;

Deionized or distilled water for dilution;

SPECIMEN COLLECTION AND STORAGE

1. Heat Inactivation: Heat inactivate samples by placing in a water bath at 56 °C for 30 min.

Note: Do not leave samples at 56 °C for longer than 1.0 h.

2. Bring samples to room temperature (20℃-25℃) before use, shake gently to mix.

3. If samples need to be stored, please store the aliquot below -20°C. Avoidrepeated freeze-thaw cycles.

Note:

a. Samples must be heat inactivated prior to use in this assay.

b. Hemolysis affects the final detection result, so hemolytic samples are not suitable for this test.

c. No detection method has been established for human plasma or whole blood samples. It is recommended that users establish their own test methods according to their needs.

REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20℃-25℃) before use.

2. As recommended in Table 2, the lyophilized materials of HRP-SARS-COV-2 Spike RBD and Calibrator (1 to 6) will be diluted into a rehydrated solution with ultrapure water/deionized water. Before use, the rehydrated solution needs to be balanced at room temperature of 30 min, shake gently every 10 min. Do not shake or vortex violently. The rehydrated solution should be stored at -70℃, Do not thaw and freeze more than 3 times.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

Catalog	Components	Amount	Stock Solution Con.	Reconstitution Buffer and Vol.
RAS044-C02	Calibrator1	0.4 mL	135.28 IU/mL	0.4 mL water
RAS044-C03	Calibrator2	0.4 mL	72.85 IU/mL	0.4 mL water
RAS044-C04	Calibrator3	0.4 mL	38.60 IU/mL	0.4 mL water
RAS044-C05	Calibrator4	0.4 mL	18.75 IU/mL	0.4 mL water
RAS044-C06	Calibrator5	0.4 mL	10.18 IU/mL	0.4 mL water
RAS044-C07	Calibrator6	0.4 mL	0 IU/mL	0.4 mL water
RAS044-C08	HRP-SARS-CoV-2 Spike RBD	15 μg	100 μg/mL	0.15 mL 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-SARS-CoV-2 Spike RBD working fluid:

Dilute HRP-SARS-CoV-2 Spike RBD rehydrated solution to 1.0 μg/mL with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

2. Add samples and Incubation

a. For Blank Control wells: P lease add 100 μL Dilution Buffer (**No add** HRP-SARS-CoV-2 Spike RBD).

b. For Calibrators and samples: Add 50μL Calibrator1-6 (**Use directly after remelting**) and samples to each well, then add 50 μL HRP-SARS-CoV-2 Spike RBD working fluid to each well;

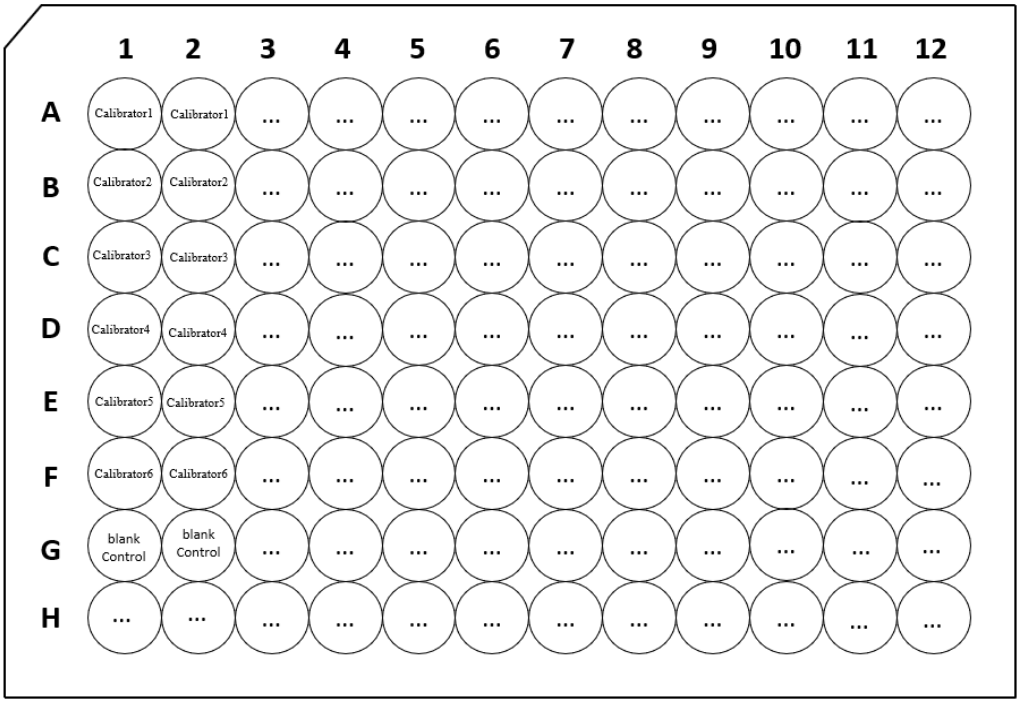
Please Seal the plate with microplate sealing film and incubate at 37℃ for 1.0 h.

Note:

a. This step needs to be operated continuously without a long interval to not to affect the results.

b. It is recommended that at least samples and Calibrators be added to double wells.

FIGURE 1. PLATE LAYOUT



3. Washing

Remove the solution from the wells by aspiration. Add 300 µL 1 x Washing Buffer to each well, gently shake the plate for 30 s. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against paper towels. Repeat the steps above for three times.

4. 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.

5. Termination

Add 50 µL **Stop Solution** to each well, shake gently to mix.

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

6. Data Recording

Read the absorbance at 450 nm、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* $OD_{630\ nm}$.

7. Data analysis:

a. Please analyze the OD value of the reading results according to the instructions of the kit. If the samples or Calibrators are added to several wells, it's necessary to calculate the average value of OD value before data analysis.

b. Establish a standard curve with linear equation. To calibrate absorbance value obtained by the calibrator curve, the OD value of the sample to be measured is subtracted to the OD value of the blank control. The calibrator curve is plotted with the calibrator concentration as x-axis and the calibrated absorbance value as y-axis. The linear regression equation was used to draw the calibration curve and calculate the concentration of samples.

LIMITATIONS OF THE PROCEDURE

1. Quality standards of Linearity: Correlation coefficient of the Calibrators curve $R^2 \geqslant 0.9900$.

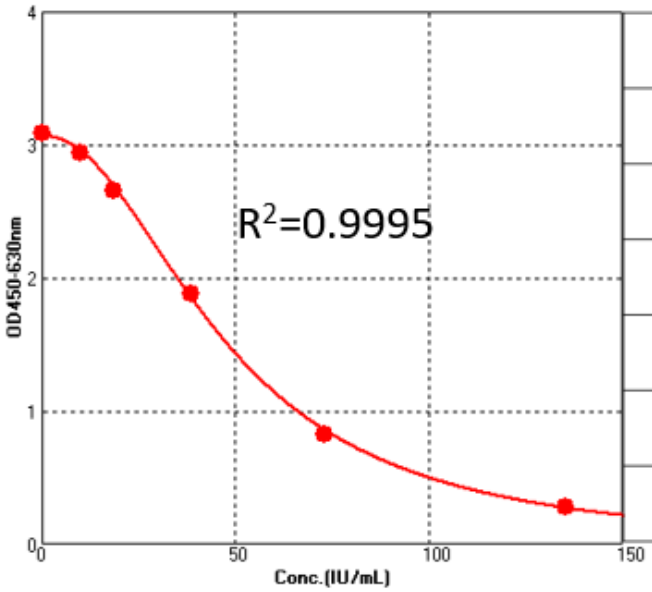
2. Detection Range: 10.18 IU/mL-135.28 IU/mL.The LoQ is 10.18 IU/mL. Values of samples are greater than the analytical measuring range should be reported as ＞135.28 IU/mL or dilute the samples so that it is within the linear range. Values of samples are less than the LOQ should be reported as <10.18 IU/mL.

PRECAUSIONS

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. All reagents should be warmed 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 10x Washing 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 calibrator curve.



(IU/mL)	O.D.-1	O.D.-2	Average
135.28	0.251	0.315	0.283
72.85	0.765	0.891	0.828
38.60	1.876	1.886	1.881
18.75	2.655	2.651	2.653
10.18	2.943	2.937	2.940
0.00	3.082	3.081	3.082