



A010-EN.01

SARS-CoV-2 Nucleocapsid Protein Titer Assay Kit

Pack Size: 96 tests

Catalog Number: RAS-A010

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 detecting SARS-CoV-2 Nucleocapsid Protein 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) is posing a serious threat to human health. A rapid and effective assay kit detecting the levels of SARS-CoV-2 Nucleocapsid Protein is urgently needed to accelerate the development of COVID-19 vaccines.

This assay kit is used to measure the levels of SARS-CoV-2 Nucleocapsid Protein by employing a standard sandwich-ELISA format. The microplate in the kit has been pre-coated with Anti-SARS-CoV-2 Nucleocapsid Protein Antibody. First add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the secondary antibody Biotin-Anti-SARS-CoV-2 Nucleocapsid 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 protein present. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm. The OD Value reflects the amount of protein bound.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RAS010-C01	Pre-coated Anti-SARS-CoV-2 Nucleocapsid Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RAS010-C02	SARS-CoV-2 Nucleocapsid Protein	10 ug	Powder	2-8°C	-70°C
RAS010-C03	Biotin-Anti-SARS-CoV-2 Nucleocapsid Antibody	100 µL	Liquid	2-8°C	2-8°C
RAS010-C04	Streptavidin-HRP	100 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS010-C05	10xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS010-C06	Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RAS010-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RAS010-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 filter;
- Centrifuge;
- 37 °C 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;

SHIPPING AND 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.
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

Bring all reagents and samples to room temperature (20°C-25°C) before use. If crystals have formed in buffer solution, warm until the crystals have completely dissolved and bring solution back to room temperature before use.

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 3 times.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

ID	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
RAS010-C02	SARS-CoV-2 Nucleocapsid Protein	10 µg	50 µg/mL	200 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 Nucleocapsid Antibody working fluid:

Dilute Biotin-Anti-SARS-CoV-2 Nucleocapsid Antibody at 1:1000 with Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

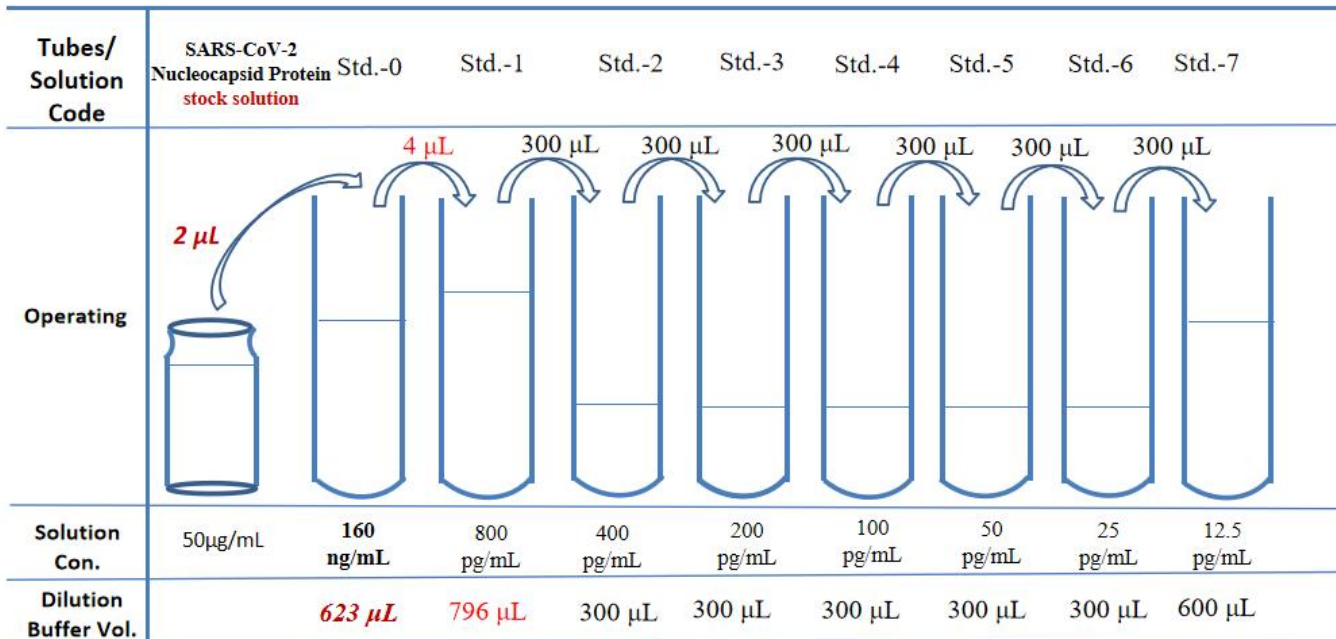
1.3 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:500 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 Nucleocapsid Protein 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 Nucleocapsid Protein



3. Add Samples

Add 100 μ L serially diluted SARS-CoV-2 Nucleocapsid Protein Standard curve (Std.-1 to Std.-7) and samples to each well. For blank Control wells, please add 100 μ L Dilution Buffer. Seal the plate with microplate sealing film and incubate at room temperature for 1.0 h.

4. Washing

Remove the remaining solution by aspiration, add 300 μ L of 1 \times Washing Buffer to each well, gently tap the plate for 30 s, 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 Nucleocapsid Antibody

For all wells, add 100 μ L Biotin-Anti-SARS-CoV-2 Nucleocapsid Antibody working solution. Seal the plate with microplate sealing film and incubate at room temperature for 1.0 h.

6. Washing

Repeat step 4.

7. Add Streptavidin-HRP

For all wells, add 100 μ L Streptavidin-HRP working solution. Seal the plate with microplate sealing film and incubate at room temperature for 1.0 h, avoid light.

8. Washing

Repeat step 4.

9. Substrate Reaction

Add 100 μ L **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 μ L **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 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

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.

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 or other statistical software 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°C-25°C) 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 standard curve.

SARS-Cov-2 Nucleocapsid Protein (pg/ml)	OD _{450 nm} -Blank
800	2.500
400	1.371
200	0.741
100	0.408
50	0.199
25	0.111
12.5	0.042

