

Version 5.0

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SARS-CoV-2 NP&RBD Total Antibody ELISA Kit

For Research Use Only. Not for Use in Diagnostic Procedures.

The operator should read technical manual carefully before using this product.

Contents

I. DESCRIPTION	2
II. BACKGROUND	2
III. ASSAY PRINCIPLE.....	2
IV. KIT CONTENTS	4
V. STORAGE.....	4
VI. REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED	4
VII. PRECAUTIONS	5
VIII. SPECIMEN COLLECTION AND STORAGE	6
IX. PROTOCOL.....	6
Reagent Preparation	6
Sample and Control Dilution.....	7
Capture Plate Preparation	7
Test Procedure.....	7
X. ASSAY PROCEDURE SUMMARY	9
XI. QUALITY CONTROL.....	10
XII. INTERPRETATION OF RESULTS.....	10
XIII. LIMITATIONS OF THE PROCEDURE	11
XIV. PRECISION	11
XV. REFERENCES.....	11
XVI. TROUBLESHOOTING	13

I. DESCRIPTION

The GenScript SARS-CoV-2 NP&RBD Total Antibody ELISA Kit is intended for the determination of total antibodies (including IgA, IgM and IgG) in human serum or plasma against SARS-CoV-2 N protein and RBD. Because of its more comprehensive detection capabilities, the kit can be used to evaluate the immune efficacy of not only mRNA COVID-19 vaccines, but also inactivated vaccines.

If you need kits to detect RBD antibodies alone, you can learn about SARS-CoV-2 Spike S1-RBD IgG&IgM ELISA Detection Kit (GenScript, L00845)

II. BACKGROUND

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, 2019-nCoV) is an enveloped non-segmented positive-sense RNA virus. It is the cause of coronavirus disease 2019 (COVID-19), which is contagious in humans.

SARS-CoV-2 has several structural proteins including spike (S), envelope (E), membrane (M) and nucleocapsid (N). The nucleocapsid protein is the most abundant protein in coronavirus. The N protein has been chosen as a diagnostic tool because of its conserved sequence and its strong immunogenicity. The spike protein is a transmembrane protein, composed of S1 and S2 subunits. The S1 subunit contains a receptor binding domain (RBD), which is responsible for recognizing the ACE2 cell surface receptor.

Infection with the SARS-CoV-2 initiates an immune response producing circulating immunoglobulin antibodies such as IgA, IgM, and IgG in blood. IgM antibody is an early indicator of the infection and IgG antibody is an important indicator of current and past infection. IgA antibody is found in mucous membranes, mainly in the respiratory and digestive tracts. It is also found in saliva, tears, breastmilk and blood.

III. ASSAY PRINCIPLE

The GenScript SARS-CoV-2 NP&RBD Total Antibody ELISA Kit is an indirect ELISA detection tool, which can be used for evaluation of total antibodies (including IgA, IgM and IgG) against SARS-CoV-2 N protein and S1-RBD in human samples. When the positive control and a

specimen are added to the capture plate, the positive control and anti-SARS-CoV-2 N protein and S1-RBD antibodies in a specimen can be captured on the plate. Other unbound molecules are removed by the washing steps. Then, detection antibody is added to the plate to detect specific anti-SARS-CoV-2 antibodies. The detection antibody is a HRP conjugated antibody, and it is an isotype independent secondary antibody recognizing human IgA, IgM, and IgG, etc. After washing steps, TMB solution is added and the color turns blue. The reaction is stopped by adding stop solution and the color turns yellow, which can be read at 450 nm by a microtiter plate reader. The absorbance of the sample depends on the titer of the anti-NP&RBD antibodies.

IV. KIT CONTENTS

Component	Quantity	Part No.
Capture Plate	1 plate	B1-80
Positive Control	1 vial (0.2 mL)	B1-10
Negative Control	1 vial (0.2 mL)	A1-11
Detection Antibody	1 bottle (12 mL)	B1-30
Sample Dilution Buffer	1 bottle (60 mL)	A1-60
20× Wash Solution	1 bottle (40 mL)	A1-70
TMB Solution	1 bottle (12 mL)	A1-40
Stop Solution	1 bottle (6 mL)	A1-50
Plate Sealer	2 pieces	N/A

- Capture Plate: 96 well microplates (8 wells x 12 strips) pre-coated with recombinant SARS-CoV-2 NP&RBD antigen; 12 strips configured in plate; Plate sealed in a foil pouch with a desiccant.

V. STORAGE

The unopened kit is stable for at least 12 months from the date of manufacture if stored at 2°C to 8°C, and the opened kit is stable for up to 1 month from the date of opening at 2°C to 8°C.

VI. REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

- Single or dual wavelength microplate reader with 450 nm filter. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.
- Automated microplate washer to wash the plate
- Deionized or distilled water to dilute 20× Wash Solution
- Graduated cylinder to prepare Wash Solution
- Plastic container to store Wash Solution
- Tubes to aliquot and dilute samples
- 10 µL, 200 µL and 1000 µL precision pipettes
- 10 µL, 200 µL and 1000 µL pipette tips

- Multichannel pipettes
- Disposable reagent reservoir
- Paper towel
- Laboratory timer
- Refrigerator to store samples and kit components
- Centrifuge
- 37 °C Incubator

VII. PRECAUTIONS

1. This product requires the handling of human specimen. It is recommended that all human - sourced materials and all consumables contaminated with potentially infectious materials will be considered potentially infectious and handled in accordance with standard precaution for infection control. Consult with your organization's health and safety department.
2. Operators should be professionally trained and have experience.
3. Do not use the kit if there is any visible damage or deviation in physical appearances of components as stated under Section IV. KIT CONTENTS.
4. Do not mix components from different batches. Do not mix with components from other manufacturers.
5. Do not use reagents beyond the stated expiration date.
6. All reagents must be allowed to equilibrate to room temperature (20°C to 25°C) before running assay. Remove only the volume of reagents that is needed. Do not pour reagents back into vials as reagent contamination may occur.
7. Before opening Positive and Negative Controls, tap the vials on the benchtop to ensure that all liquid or powder is at the bottom of the vial.
8. Use only distilled or deionized water and clean glassware.
9. Do not let wells dry during the test, add reagents immediately after completing washing steps.
10. Decontaminate and dispose of all specimens, controls, reagents, and other potentially

contaminated materials in accordance with local, state, and federal regulations.

11. All materials should be handled in a manner that minimizes the chance of potential contamination of the work area.

VIII. SPECIMEN COLLECTION AND STORAGE

1. Handle all blood and serum as if capable of transmitting infectious agents.
2. The NCCLS provides recommendations for handling and storing serum and plasma specimens (Approved Standard- Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).
3. No prior special preparation is required before sample collection by approved techniques. Collect the specimen in accordance with normal laboratory practice. Specimens should be collected aseptically by venipuncture. Early separation from the clot prevents hemolysis of serum.
4. Do not use haemolysed, clotted, contaminated and viscous specimen. Specimen containing particulate matter should be centrifuged.
5. Store specimens at -20°C or lower if not tested immediately. Avoid repeated freeze-thaw cycles.
6. The handling and storage information provided here is based on references maintained by the manufacturer. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria to meet specific needs.

IX. PROTOCOL

● Reagent Preparation

1. All reagents must be removed from refrigeration and allowed to return to room temperature before use (20°C to 25°C). Save all reagents in refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. 1× Wash Solution Preparation: Dilute the 20× Wash Solution with deionized or distilled

water with a volume ratio of 1:19. For example, dilute 40 mL of 20× Wash Solution with 760 mL of deionized or distilled water to make 800 mL of 1× Wash Solution. Store the solution at 2°C to 8°C when not in use.

Note: If any precipitate is found in the 20× Wash Solution, incubate the bottle in a water bath (up to 50°C) with occasionally mixing until all the precipitate is dissolved.

● Sample and Control Dilution

Dilute test samples, Positive, and Negative Controls with a 1:100 dilution ratio with Sample Dilution Buffer. For each 5 µL of sample, 495 µL of Sample Dilution Buffer is needed. Samples also can be further serially diluted if relative quantification is needed by the user.

● Capture Plate Preparation

1. It is recommended that all Positive Control, and Negative Control should be prepared in duplicate.
2. Count the strips according to the number of test samples and install the strips. Make sure the strips are tightly snapped into the plate frame.

Test Configuration

	1	2	3	4	5	6	7	8	9	10	11	12
A	Negative Control											
B	Negative Control											
C	Positive Control											
D	Positive Control											
E												
F												
G												
H												

3. Leave the unused strips in the foil pouch and store at 2°C to 8°C. The strips must be stored in the closed foil pouch to prevent moisture from damaging the Capture Plate.

● Test Procedure

Positive Control, Negative Control and Samples Incubation

1. Add 100 µL of diluted Positive Control, diluted Negative Control, and the samples to the corresponding wells.

2. Cover the plate with Plate Sealer and incubate at 37°C for 30 minutes.
3. Remove the Plate Sealer and wash the plate with 260 µL of 1× Wash Solution for four times.
4. Pat the plate on paper towel to remove residual liquid in the wells after washing steps.

Detection Antibody Incubation

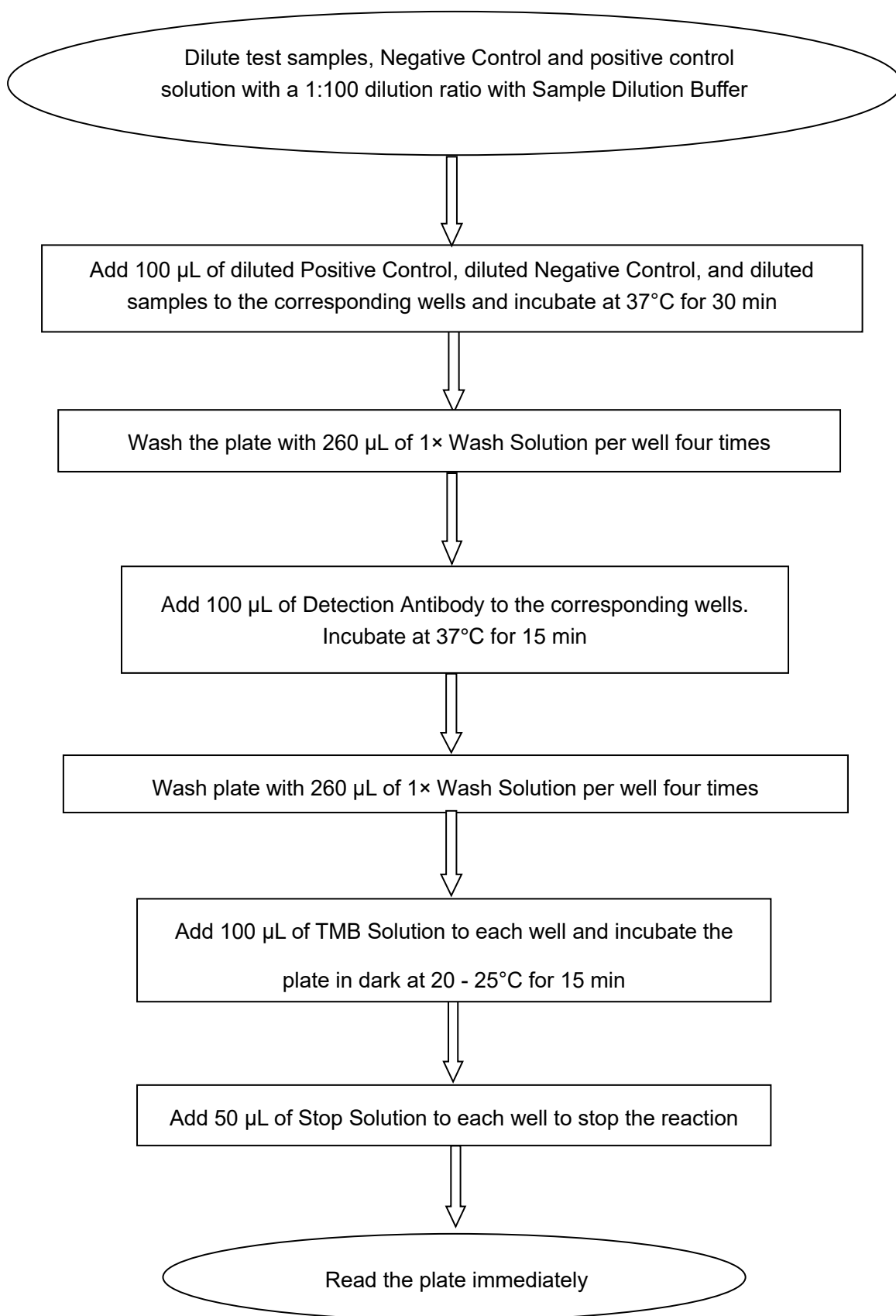
5. Add 100 µL of Detection Antibody to each well.
6. Cover the plate with Plate Sealer and incubate at 37°C for 15 minutes.
7. Remove the Plate Sealer and wash the plate with 260 µL of 1× Wash Solution for four times.
8. Pat the plate on paper towel to remove residual liquid in the wells after washing steps.

Substrate Reaction and Absorbance Measurement

9. Add 100 µL of TMB Solution to each well and incubate the plate in dark at 20 - 25°C for 15 minutes (Start timing after the addition of TMB Solution to the first well).
10. Add 50 µL of Stop Solution to each well to stop the reaction.
11. Read the absorbance in microtiter plate reader set to 450 nm immediately.

Note: The substrate reaction time is determined by the temperature. The ideal reaction temperature is 25°C. If the temperature is below 25°C, extend the reaction time appropriately.

X. ASSAY PROCEDURE SUMMARY



XI. QUALITY CONTROL

To assure the validity of the results, each assay must include both Positive and Negative Controls. The average optical density (OD450) of control must fall within the ranges listed in the following table. If OD450 values of controls do not meet the requirements in the following table, the test is invalid and must be repeated.

- OD450 values for quality control

Items	OD450 value	Control for Valid Assay
Quality Control	< 0.1	Negative Control tested by Detection Antibody
	≥ 0.8	Positive Control tested by Detection Antibody

Note: The standards in the table are only intended to evaluate the performance of the kit.

XII. INTERPRETATION OF RESULTS

The results of the GenScript SARS-CoV-2 NP&RBD Total Antibody ELISA Kit are calculated by the sample OD450 value (S) and the Cutoff value (C.O.).

Calculation of the Cutoff value (C.O.) = the average optical density (OD450) of Negative Control + 0.12.

The operator can determine the result of the sample by comparing the S/CO* to the following table.

Items	S/CO	Result	Interpretation
SARS-CoV-2 IgG test	< 1	Negative	SARS-CoV-2 antibodies not detected
	≥ 1	Positive	SARS-CoV-2 antibodies detected

*The S/CO is based on testing with our panel of confirmed COVID-19 sera samples and healthy control sera samples. Users may want to set up their own standard based on different serum samples from different geographic locations or different ethnic backgrounds.

For positive samples, if the user needs a relative quantification of the antibodies, the value of S/CO can be used to analyze. For example, if the calculated S/CO value is < 5, it means that the level of antibodies in the sample is medium-low, if $5 \leq \text{S/CO} \leq 10$, it means the antibody level is medium-high, and if $\text{S/CO} > 10$, it means the antibody level is high.

XIII. LIMITATIONS OF THE PROCEDURE

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1. This test is designed for qualitative detection.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the manual is necessary to obtain reliable test results.
3. A negative result can occur if the titer of antibodies against the SARS-CoV-2 virus present in the samples is below the sensitivity of the kit.
4. If symptoms persist and the result from the SARS-CoV-2 antibody test is negative, it is recommended to collect a new sample from the patient a few days later and test it again.

XIV. PRECISION

- Intra-assay: One known level of control was spiked into sample buffer as a test sample. The sample was tested 10 times on the same plate to evaluate intra-assay precision of the kit. Intra-assay variation of this kit is less than or equal to 10%.
- Inter-assay: One known level of control was spiked into sample buffer as a test sample. The sample was tested on 3 plates which were randomly selected from 3 different lots to evaluate inter-assay precision of the kit. Inter-assay variation of this kit is less than or equal to 15%.

XV. REFERENCES

1. Chinese Center for Disease Control and Prevention (2020) Public protection guidelines for Novel coronavirus pneumonia, People's Medical Publishing House (PMPH).
2. ZHOU Peng, YANG Xinglou. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature, 2020.
3. XUE Xiongyan, ZHU Changlin, HUANG Shaozhen, Inactivation of 2019 new coronary virus before antibodies detection by different methods. Journal of Southern Medical University, 2020.
4. SHI Heshui, HAN Xiaoyu, FAN Yanqing. Radiologic Features of Patients with 2019-n Co V Infection. Journal of Clinical Radiology, 2020.

5. NCCLS. 1991. National Committee for Clinical Laboratory Standard. Internal Quality
6. Testing of Reagent Water in the Clinical Laboratory. NCCLS Publication C3-A3.
7. NCCLS. 1997. National Committee for Clinical Laboratory Standard. Preparation and Testing of Reagent Water in the Clinical Laboratory. NCCLS Publication C3-A3.

XVI. TROUBLESHOOTING

Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Wells are scratched with pipette tip or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates are found in the samples	Remove any particulates by centrifugation prior to the assay
Weak/No Signal	Substrate is not added or added at the wrong time	Follow the manual to add the substrate properly
	Components are used from other lots or sources	Use only lot-specific components
	Substrate is contaminated	Use new Substrate with same Lot
	Volumes of reagents are not correct	Repeat assay with the required volumes in manual
	The plate is not incubated for proper time or temperature	Follow the manual to repeat assay
	The plate is not read within the specified time range	Read the plate within 5 minutes
High Background	Plate is not washed properly	Make sure the wash apparatus works properly
	Substrate is contaminated	Use new substrate with same Lot
	Evaporation of wells during incubations	Perform incubation steps with plate sealer in repeat assay
	Incorrect incubation times and/or temperatures	Follow the manual to repeat the assay