Version 3.1
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SARS-CoV-2 NP&RBD Total Antibody ELISA Detection Kit

Cat. No. : L00846 96 Tests

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

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 Tel: 1-732-885-9188

Contents

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I. INTENDED USE

The GenScript SARS-CoV-2 NP&RBD Total Antibody ELISA Detection Kit is intended for the determination of total antibodies (including IgA, IgM and IgG) in human serum or plasma against SARS-CoV-2 Spike NP&RBD recombinant antigen.

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 Detection 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

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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 dependents on the titer of the anti-NP&RBD antibodies.

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IV. KIT CONTENTS

Component	Quantity	Part No.
Capture Plate	1 plate	B1-80
Positive Control	1 vial (0.05 mL)	B1-10
Negative Control	1 vial (0.05 mL)	B1-11
Detection Antibody	1 bottle (12 mL)	B1-30
Sample Dilution Buffer	1 bottle (60 mL)	B1-60
20× Wash Solution	1 bottle (40 mL)	B1-70
TMB Solution	1 bottle (12 mL)	B1-40
Stop Solution	1 bottle (6 mL)	B1-50
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
- 2 µL/2.5 µL, 10 µL, 200 µL and 1000 µL precision pipettes
- 2 µL/2.5 µL, 10 µL, 200 µL and 1000 µL pipette tips

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

VII. PRECAUTIONS

1. Although this product itself does not contain any material or reagent that can cause infection, the blood or sera collected from SARS-CoV-2 patients (whether newly infected or recovered), and “uninfected” people (could have other potential infectious agents) must be handled with high level of precaution.
2. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents should be handled at the Biosafety Level 2 facility.
3. Do not mix components from different batches. Do not mix with components from other manufacturers.
4. Do not use reagents beyond the stated expiration date.
5. 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.
6. Before opening the Positive and Negative Controls, tap the vials on the benchtop to ensure that all liquid is at the bottom of the vial.
7. Use only distilled or deionized water and clean glassware.
8. Do not let wells dry during assay; add reagents immediately after completing wash steps.

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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. For performance of the GenScript SARS-CoV-2 NP&RBD total antibody ELISA detection, a minimum volume of 10 μL per non-hemolyzed serum or plasma sample is recommended, in case that repeat testing is required. Specimens should be collected aseptically by venipuncture. Early separation from the clot prevents hemolysis of serum.
4. For human serum, use a blood separator tube and allow sample to clot for 30 minutes, then centrifuge for 10 minutes at 1000 g. Run assay immediately, otherwise store aliquot below -20°C. Avoid repeated freeze-thaw cycles.
5. For human plasma, treat blood with an anticoagulant such as citrate, EDTA or heparin. Centrifuge for 10 minutes at 1000 g within 30 minutes for plasma collection. Run assay immediately, otherwise store aliquot below -20°C. Avoid repeated freeze-thaw cycles.

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.

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- **Sample and Control Dilution**

  Dilute test samples, Positive, and Negative Controls with a 1:100 dilution ratio with Sample Dilution Buffer. For each 1.5 µL of sample, 148.5 µL of Sample Dilution Buffer is needed.

Note: we recommend the usage of small volume pipette such as P2 or P2.5 pipette for dispensing such small sample volume to maintain accuracy.

- **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

	Example	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

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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 10-15 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℃. If the temperature is below 25℃, extend the reaction time appropriately.

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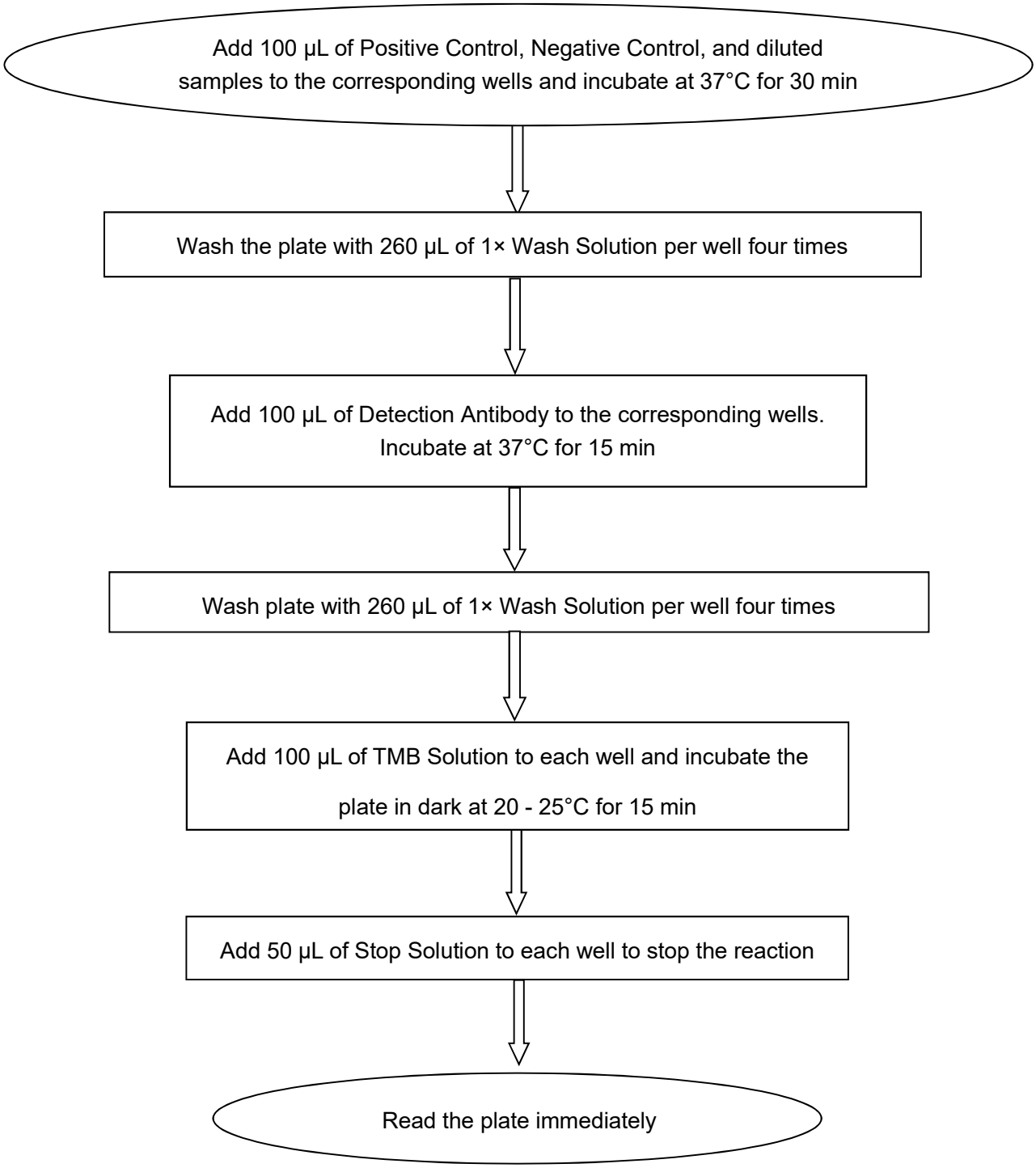
X. ASSAY PROCEDURE SUMMARY



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XI. QUALITY CONTROL

To assure the validity of the results, each assay must include both Positive and Negative Controls. The net 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	< 0.13	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 positive and negative cutoff values for SARS-CoV-2 NP&RBD total antibody detection can be used for interpretation of the sample OD values. The operator can determine the result of the sample by comparing the OD to the following table.

- Cutoff Interpretation*

Items	Cutoff OD 450	Result	Interpretation
SARS-CoV-2 IgG test	< 0.13	Negative	No detectable SARS-CoV-2 antibody.
	0.13 - 0.15	Borderline	Testing should be repeated by an alternative method or another sample should be collected.
	> 0.15	Positive	SARS-CoV-2 antibody detected.

*The cutoff value is based on validation with our panel of confirmed COVID-19 patient sera and healthy control sera. Users may want to set up their own cutoff based on different patient serum panels from different geographic locations or different ethnic backgrounds.

XIII. LIMITATIONS OF THE PROCEDURE

This test is designed for qualitative detection.

1. The user of this kit is advised to carefully read and understand the package insert. Strict

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adherence to the manual is necessary to obtain reliable test results.

2. A negative result can occur if the titer of antibodies against the SARS-CoV-2 virus present in the specimen is below the sensitivity of the kit.

3. 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. All samples were 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. All samples were tested in 6 separate assays to evaluate intra-assay precision of the kit. Inter-assay variation of this kit is less than or equal to 15%.

XV. CLINICAL PERFORMANCE

Serum samples from a cohort of patients were tested using the NP&RBD total antibody test kit. The combined cohort consisted of samples from normal healthy people (n=83) and samples from RT-PCR confirmed SARS-CoV-2 positive patients (n=54).

Method		**RT-PCR Test**	**Healthy sample**
		Positive (n=54)	**Negative (n=83)**
SARS-CoV-2 NP&RBD Total Antibody ELISA Detection Kit	Positive	51	0
	Negative	3	83
	Sensitivity*	94.4%	
	Specificity		100%

*It is important to note that not all PCR positive COVID-19 patients seroconvert.

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XVI. REFERENCES

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XVII. 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

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