Version 4.2
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SARS-CoV-2 Spike S1-RBD IgG&IgM ELISA Detection Kit

Cat. No. : L00845	96 Tests
Cat. No. : L00845-5	480 Tests

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

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Contents

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I. INTENDED USE

The GenScript SARS-CoV-2 Spike S1-RBD IgG&IgM ELISA Detection Kit is intended for the determination of IgG and IgM antibodies separately against SARS-CoV-2 Spike S1-Receptor Binding Domain (RBD) in human serum or plasma.

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 spike protein (S) 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 cell surface receptor, angiotensin converting enzyme-2 (ACE2). It is found that the RBD of the SARS-CoV-2 S protein strongly interacts with the human ACE2 receptor leading to endocytosis into the host cells of the deep lung, thus leading to viral replication.

Infection with the SARS-CoV-2 initiates an immune response producing circulating immunoglobulin antibodies IgM and IgG. The IgM antibody is an early indicator of the infection and the IgG antibody is an important indicator of current and past infection.

III. ASSAY PRINCIPLE

The GenScript SARS-CoV-2 Spike S1-RBD IgG&IgM ELISA Detection Kit is an indirect ELISA detection tool, which can be used for evaluation of anti-SARS-CoV-2 Spike S1-RBD IgG or IgM in human samples. A purified recombinant SARS-CoV-2 Spike S1-RBD antigen is bound to the wells of a Capture Plate. A horseradish peroxidase (HRP) coupled anti-human IgG conjugate is used for IgG determination. Similarly, a HRP conjugated mouse anti-Human IgM is used for IgM determination.

When the Positive control, Negative Control, and specimen are added to capture plates, the positive control and SARS-CoV-2 spike protein S1-RBD antibodies in specimen can be

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captured on the plate. Other unbound molecules are removed by the washing steps. Then, the HRP conjugated Mouse anti-Human IgG or HRP conjugated mouse anti-human IgM is added to the plate. 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-S1-RBD protein antibodies.

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IV. KIT CONTENTS

Component	96 Tests		480 Tests	
	Quantity	Part No.	Quantity	Part No.
Capture Plate	1 plate	A1-80	5 plates	A5-80
Positive Control	1 vial (0.05 mL)	A1-10	1 vial (0.25 mL)	A5-10
Negative Control	1 vial (0.05 mL)	A1-11	1 vial (0.25 mL)	A5-11
HRP conjugated Mouse anti-Human IgG	1 bottle (12 mL)	A1-30	1 bottle (60 mL)	A5-30
HRP conjugated Mouse anti-Human IgM	1 bottle (12 mL)	A1-31	1 bottle (60 mL)	A5-31
Sample Dilution Buffer	1 bottle (60 mL)	A1-60	3 bottles (300 mL)	A5-60
20× Wash Solution	1 bottle (40 mL)	A1-70	2 bottles (200 mL)	A5-70
TMB Solution	1 bottle (12 mL)	A1-40	1 bottle (60 mL)	A5-40
Stop Solution	1 bottle (6 mL)	A1-50	1 bottle (30 mL)	A5-50
Plate Sealer	2 pieces	N/A	10 pieces	N/A

- Capture Plate: 96 well microplates (8 wells × 12 strips) pre-coated with recombinant SARS-CoV-2 Spike S1-RBD antigen; 12 strips configured in plate; Plate sealed in a foil pouch with a desiccant.
- Positive Control: Containing a monoclonal IgG specific for SARS-CoV-2, a monoclonal IgM specific for SARS-CoV-2.

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.

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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
- 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 materials or reagents that can cause infection, the blood or sera collected from SARS-CoV-2 patients (whether newly infected or recovered), and “uninfected” people (which could have other potential infectious agents) must be handled with a 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.

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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 Positive Control and Negative Control, 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 the test, add reagents immediately after completing washing steps.

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 Spike S1-RBD IgG&IgM ELISA, 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.

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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 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 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.

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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. If you do analysis of the IgG and IgM samples on the same plate. Load controls twice. An detect one set with anti-IgG and second set with anti-IgM antibody
4. 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 Sample 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 a paper towel to remove residual liquid in the wells after washing steps.

Note: If you want to measure IgG and IgM simultaneously, use two wells for each sample, one well for IgG, and one well for IgM.

HRP conjugated Mouse anti-Human IgG/ HRP conjugated Mouse anti-Human IgM Incubation

5. Add 100 µL of HRP conjugated Mouse anti-Human IgG or HRP conjugated Mouse anti-Human IgM 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

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four times.

8. Pat the plate on paper towel to remove residual liquid in the wells after washing steps.

Note: Please choose the secondary antibody based on the type of antibodies to be determined. For example, HRP conjugated mouse anti-human IgG is used for IgG detection and HRP conjugated mouse anti-human IgM is used for IgM detection.

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 quench the reaction.
11. Read the absorbance in microtiter plate reader at 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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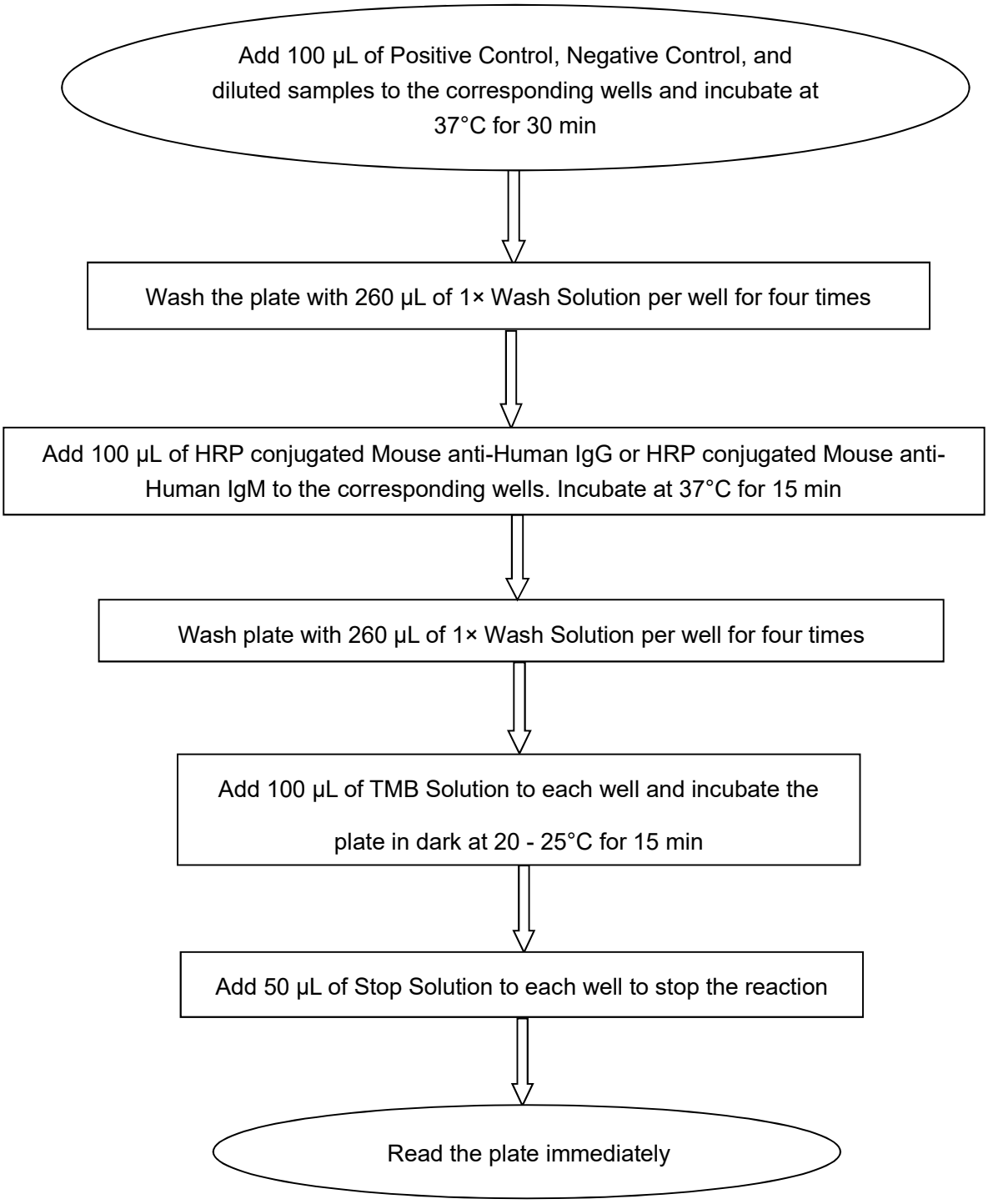
X. ASSAY PROCEDURE SUMMARY



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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**	**Test for Valid Assay**
SARS-CoV-2 IgG test	Negative Control	< 0.16	Negative Control tested with HRP conjugated Mouse anti-Human IgG
	Positive Control	≥ 1.0	Positive Control tested with HRP conjugated Mouse anti-Human IgG
SARS-CoV-2 IgM test	Negative Control	< 0.16	Negative Control tested with HRP conjugated Mouse anti-Human IgM
	Positive Control	≥ 0.80	Positive Control tested with HRP conjugated Mouse anti-Human IgM

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

XII. INTERPRETATION OF RESULTS

The positive cutoff and negative cutoff for SARS-CoV-2 IgG and IgM detection can be used for interpretation of the sample OD450 values. The operator can determine the result of the sample by comparing the OD to the following table.

- Cutoff Interpretation*

Items	**Cutoff OD450**	**Result**	**Interpretation**
SARS-CoV-2 IgG test	< 0.16	Negative	No detectable SARS-CoV-2 IgG antibody
	0.16 - 0.2	Borderline	Testing should be repeated by an alternative method or another sample should be collected
	> 0.2	Positive	SARS-CoV-2 IgG antibody detected
SARS-CoV-2 IgM test	< 0.16	Negative	No detectable SARS-CoV-2 IgM antibody
	0.16 - 0.2	Borderline	Testing should be repeated by an alternative method or another sample should be collected
	> 0.2	Positive	SARS-CoV-2 IgM antibody detected

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*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 their own cutoff based on different patient serum panels from different geographic locations or different ethnic backgrounds.

- Relationship between Anti-SARS-CoV-2 Antibody and COVID-19

		SARS-CoV-2 IgM Test	
		Positive	**Negative**
SARS-CoV-2 IgG Test	**Positive**	Indicates active COVID-19	Indicates current and past infection of COVID-19
	Negative	Indicates early infection of COVID-19	Indicates no antibody response

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. Following the protocol as indicated in the manual is necessary to obtain reliable results. Changing the protocol may result in unreliable 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 GenScript SARS-CoV-2 Spike S1-RBD IgG&IgM ELISA Detection Kit is negative, it is recommended to collect another 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%.

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XV. CLINICAL PERFORMANCE

Serum and plasma samples from a cohort of patients were tested using the IgG&IgM ELISA kit. The combined cohort consisted of samples from normal healthy people (n=98) and samples from RT-PCR confirmed SARS-CoV-2 positive patients (n=69).

Method			RT-PCR Test	Healthy sample
			Positive (n=69)	Negative (n=98)
SARS-CoV-2 Spike S1-RBD IgG&IgM ELISA Detection Kit	Positive	IgG+ or IgM+	65	0
	Negative	IgG-/IgM-	4	98
	Sensitivity*		94.2%	
	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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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 Testing of Reagent Water in the Clinical Laboratory. NCCLS Publication C3-A3.
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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 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
	Plate 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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