Version RUO 5.0
Update: 09/18/2020

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

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

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

Contents

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

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.

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	A1-10	1 vial	A5-10
Negative Control	1 vial (0.2 mL)	A1-11	1 vial (1 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); 12 strips configured in plate sealed in a foil pouch with a desiccant.
- Positive Control: White freeze-dried powder; Containing IgG and IgM antibodies specific to SARS-CoV-2.

V. STORAGE

The unopened kit is stable for at least 12 months from the date of manufacture if stored at 2 to 8°C, and the opened kit is stable for up to 1 month from the date of opening at 2 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
- 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 towels
- Laboratory timer
- Refrigerator to store samples and kit components
- Centrifuge
- 37 ºC 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.
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 taken out from refrigeration and returned to room temperature before use (20 to 25°C). Save all reagents in refrigerator promptly after use.
2. Reconstitute the lyophilized Positive Control with 200 µL of deionized water (or equivalent). Put the bottle face up for 5 min, and then shake up-down slowly for 3 - 5 times, place it upside down for 5 min. Avoid bubbles when shaking. The positive control solution is clear and colorless.
3. All samples and controls should be vortexed before use.
4. 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 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, Negative Control and positive control solution with a 1:100 dilution ratio with Sample Dilution Buffer. For each 5 µL of sample, 495 µL of Sample Dilution Buffer is needed.

- **Capture Plate Preparation**

1. It is recommended that all Positive Controls, and Negative Controls should be prepared in duplicate.
2. Count the strips for the assay according to the test configuration and 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. If you do analysis of the IgG and IgM samples on the same plate. Load controls twice. A 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 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. Tap 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

four times.

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

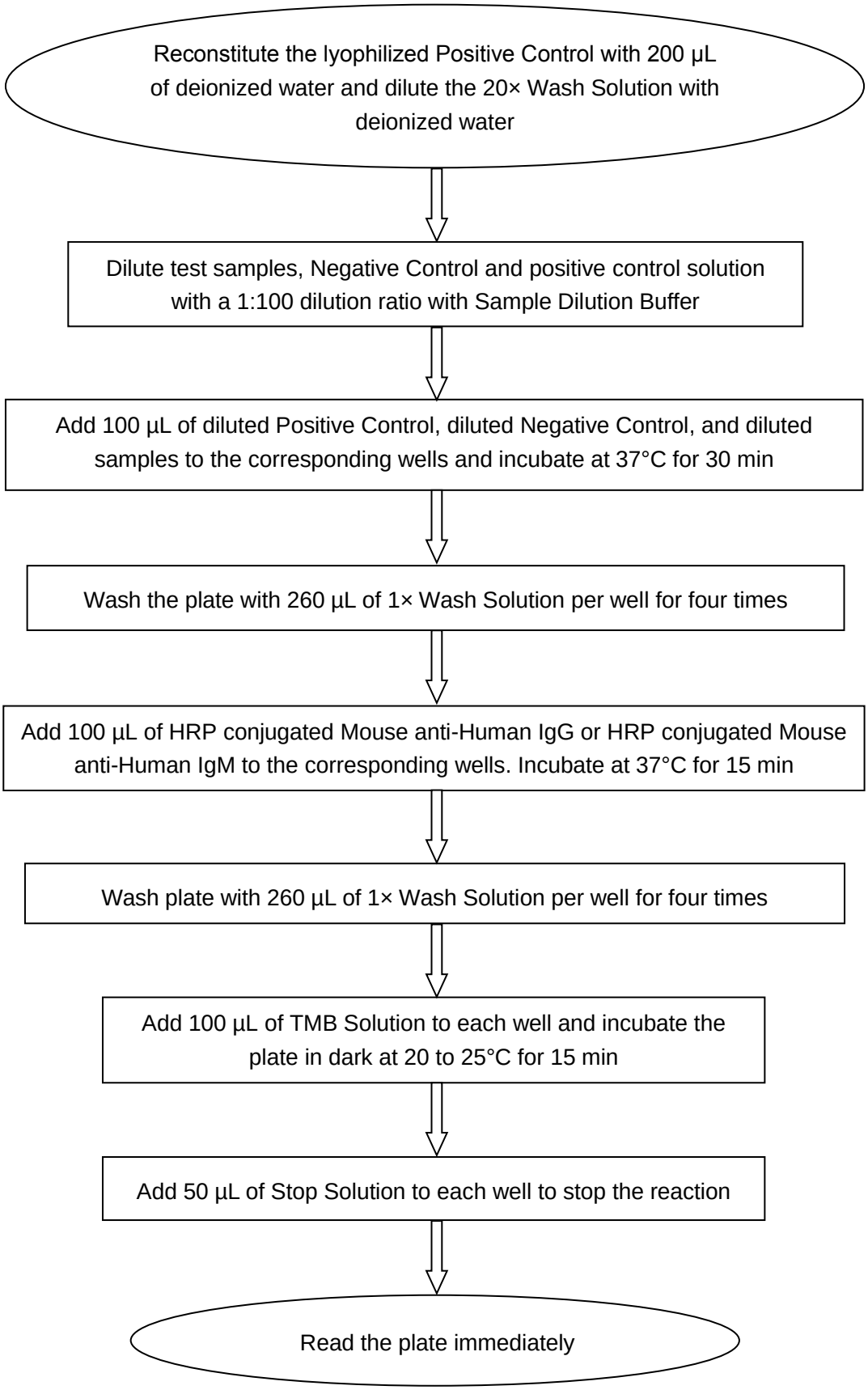
Note: Please choose the secondary antibody based on the type of antibodies to be determined. For example, HRP conjugated mouse anti-human IgG is used for IgG detection and HRP conjugated mouse anti-human IgM is used for IgM detection.

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°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**
Negative Control tested with HRP conjugated Mouse anti-Human IgG or HRP conjugated Mouse anti-Human IgM	< 0.1
Positive Control tested with HRP conjugated Mouse anti-Human IgG or HRP conjugated Mouse anti-Human IgM	≥ 0.6

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 Spike S1-RBD IgG&IgM ELISA Detection 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 IgG antibodies not detected
	≥ 1	Positive	SARS-CoV-2 IgG antibodies detected
SARS-CoV-2 IgM test	< 1	Negative	SARS-CoV-2 IgM antibodies not detected
	≥ 1	Positive	SARS-CoV-2 IgM antibodies detected

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

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.
3. Following the protocol as indicated in the manual is necessary to obtain reliable results. Changing the protocol may result in unreliable results.
4. 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.
5. 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%.

XV. CLINICAL PERFORMANCE

- Positive Percent Agreement (PPA)

  The positive percent agreement (PPA) of the GenScript SARS-CoV-2 Spike S1-RBD IgG&IgM ELISA assay was determined by testing serum samples collected from 20 RT-PCR confirmed SARS-CoV-2 positive patients. The positive percent agreement (PPA) was 100%.

- Negative Percent Agreement (NPA)

  A total of 81 serum samples from normal human were tested using the GenScript SARS-CoV-2 Spike S1-RBD IgG&IgM ELISA kit. No false positive sample was detected. The negative percent agreement (NPA) was 100%.

Method			**RT-PCR Positive (n=20)**	**Normal Human (n=81)**
SARS-CoV-2 Spike S1-RBD IgG&IgM ELISA Detection Kit	Positive	IgG+ or IgM+	20	0
	Negative	IgG-/IgM-	0	81
	PPA		100%(20/20)	
	NPA			100%(81/81)

XVI. 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 Testing of Reagent Water in the Clinical Laboratory. NCCLS Publication C3-A3.
6. NCCLS. 1997. National Committee for Clinical Laboratory Standard. Preparation and Testing of Reagent Water in the Clinical Laboratory. NCCLS Publication C3-A3.

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