Version 1.0
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SARS-CoV-2 Spike S1-RBD IgG ELISA Detection Kit

Cat. No. L00830

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

Research use only. Not for diagnostic use.

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Contents

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I. DESCRIPTION

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 structure proteins including spike (S), envelope (E), membrane (M) and nucleocapsid (N). The spike protein (S) is a transmembrane protein, composed of S1 subunit and S2 subunit. S1 subunit contains a receptor binding domain (RBD), which is responsible for recognizing the cell surface receptor. It is found that the RBD domain of the SARS-CoV-2 S protein strongly interacts with human ACE2 receptor, causing infection of the human respiratory epithelial cells.

When human infects with the SARS-CoV-2, it initiates an immune response in human body. After infection immunoglobulin antibodies such as IgM and IgG appear in blood. IgM antibody is an early indicator of the infection and IgG antibody is an important indicator of recent and past infection.

SARS-CoV-2 Spike S1-RBD IgG ELISA Detection Kit is an indirect ELISA detection tool which can be used for evaluation of anti-SARS-CoV-2 Spike S1-RBD IgG in samples. When the positive control and specimen are added to capture plate, which has been pre-coated with recombinant SARS-CoV-2 spike protein S1-RBD, 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, HRP conjugated mouse anti-human IgG Fc is added to the plate. After washing steps, TMB solution is added and the color turns blue. The reaction will be 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 is proportional to the concentration of the anti-S1-RBD protein IgG.

The result is not used as a clinical diagnosis basis.

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II. KEY FEATURES

Features	Specification
Rules for IgG evaluation	OD values of samples compared with ones of a weak positive standard (0.008 μg/mL) and a strong positive standard (1 μg/mL)
Precision	Inter-assay: ≤ 10% Intra-assay: ≤ 15%
Test Samples	Human serum/ plasma
Conveniency	All reagents and buffers for test are provided complete the test within 1 hours

III. KIT CONTENTS

- Reagents and buffers for SARS-CoV-2 Spike S1-RBD IgG detection.

Component	Quantity	Part No.
Capture Plate	1 plate (8 wells × 12 strips)	L00830-80
IgG Positive Control (25 μg/ml)	1 mL	L00830-10
HRP conjugated Mouse anti-Human IgG Fc	12 mL	L00830-30
Sample Dilution Buffer	20 mL	L00830-60
20× Wash Solution	40 mL	L00830-70
TMB Solution	12 mL	L00830-40
Stop Solution	6 mL	L00830-50
Plate Sealer	2 pieces	N/A
User Manual	1 copy	N/A

IV. STORAGE

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

Note: Do not mix the kit components of reagents among different batches, and only use the kit components within the same batch for each set of experiments.

V. REAGENTS/EQUIPMENT BUT NOT SUPPLIED

Microtiter plate reader capable of measuring absorbance at 450 nm

Automated microplate washer to wash the plate

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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, 100 µL, 200 µL and 1000 µL precision pipettes

10 µL, 100 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

VI. PROTOCOL

- All reagents in the kit and test samples should be equilibrated to room temperature before test.
- Preliminary experiment should be performed to optimize the sample dilution.

Reagent Preparation

- **1× Wash Solution Preparation:** Dilute *20× Wash Solution* by 1:19 v/v with deionized or distilled water. 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 at 2-8°C.

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

- **Positive Control Standards Preparation**

  The kit provides IgG antibody control for sample test.

1. Label six 1.5 mL Eppendorf tubes with '5 µg/mL', '1 µg/mL', '0.2 µg/mL', '0.04 µg/mL', '0.008 µg/mL', '0 µg/mL'.
2. Pipette 400 µL of *Sample Dilution Buffer* into six empty tubes.
3. Pipette 100 µL of 25 µg/mL of *IgG Positive Control* to the tube labeled with '5 µg/mL' and vortex it to make the standard be 5 µg/mL.

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4. Pipette 100 µL of 5 µg/mL of IgG Positive Control solution to the tube labeled with '1 µg/mL' and vortex it to make the standard be 1 µg/mL.
5. Similarly prepare the rest of the standard solution series (0.2, 0.04, 0.008µg/mL).

Samples Preparation

Handle serum or plasma samples in accordance with NCCLS (National Committee for Clinical Laboratory Standards) guidelines for preventing transmission of blood - bore infection.

- **Human Serum:** Use a blood separator tube and allow sample to clot for 30 minutes, then centrifuge for 10 minutes at 1000g. Run assay immediately, otherwise store aliquot sample below -20°C. Avoid repeat freeze-thaw cycle. When the human serum is tested, it should be diluted 100 fold at least.
- **Human Plasma:** Treat blood with anticoagulant such as citrate, EDTA or heparin. Centrifuge for 10 minutes at 1000g within 30 minutes for plasma collection. Run assay immediately, otherwise store aliquot sample below -20°C. Avoid repeat freeze-thaw cycle. When the human plasma is tested, it should be diluted 100 fold at least.
- Perform preliminary experiment to determine the optimum detection sample dilution.

The standard curve and sample dilution design in the following table

	Positive Controls (µg/mL)		Samples Dilution									
	Duplicate 1	Duplicate 2	Sample 1	Sample 1	5	6	7	8	9	10	11	12
A	25.00	25.00	Non-diluted	Non-diluted								
B	5.00	5.00	1/100	1/100								
C	1.00	1.00	1/1000	1/1000								
D	0.20	0.20	1/10000	1/10000								
E	0.04	0.04										
F	0.008	0.008										
G	0	0										
H	Blank	Blank										

Capture Plate Preparation

- It is recommended that all Positive Control Standards and samples be prepared in duplicate.

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- Count the strips for the assay and make sure the strips are tightly snapped in the plate frame.
- Leave the unused strips in the foil pouch and store at 2-8°C. The strips must be stored in the closed foil pouch to prevent moisture because the moisture can damage the *Capture Plate*.

Test Procedure

- **Positive Control Standards and Samples Incubation**
  1. Add 100 µL of a set of Positive Control Standards and 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 a*nd 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 wash steps.
- **HRP conjugated Mouse anti-Human IgG Fc Incubation**
  1. Add 100 µL of *HRP conjugated Mouse anti-Human IgG Fc* to each well.
  2. Cover the plate with *Plate Sealer* and incubate at 37°C for 15 minutes.
  3. Remove the *Plate Sealer* and wash the plate with 260 µL of 1× Wash Solution for five times.
  4. Pat the plate on paper towel to remove residual liquid in the wells after wash steps.
- **Substrate Reaction and Absorbance Measurement**
  1. Add 100 µL of *TMB Solution* to each well and incubate the plate in dark at 20-25°C for 10-15 minutes (from the *TMB Solution* were added to the first well to start timing).
  2. Add 50 µL of *Stop Solution* to each well to stop the reaction.
  3. Read the absorbance in microtiter plate reader set to 450 nm immediately.

Note: The substrate reaction time is determined by the temperature, the perfect reaction temperature is 25 ℃.When the temperature is below 25 ℃, appropriate extend the reaction time.

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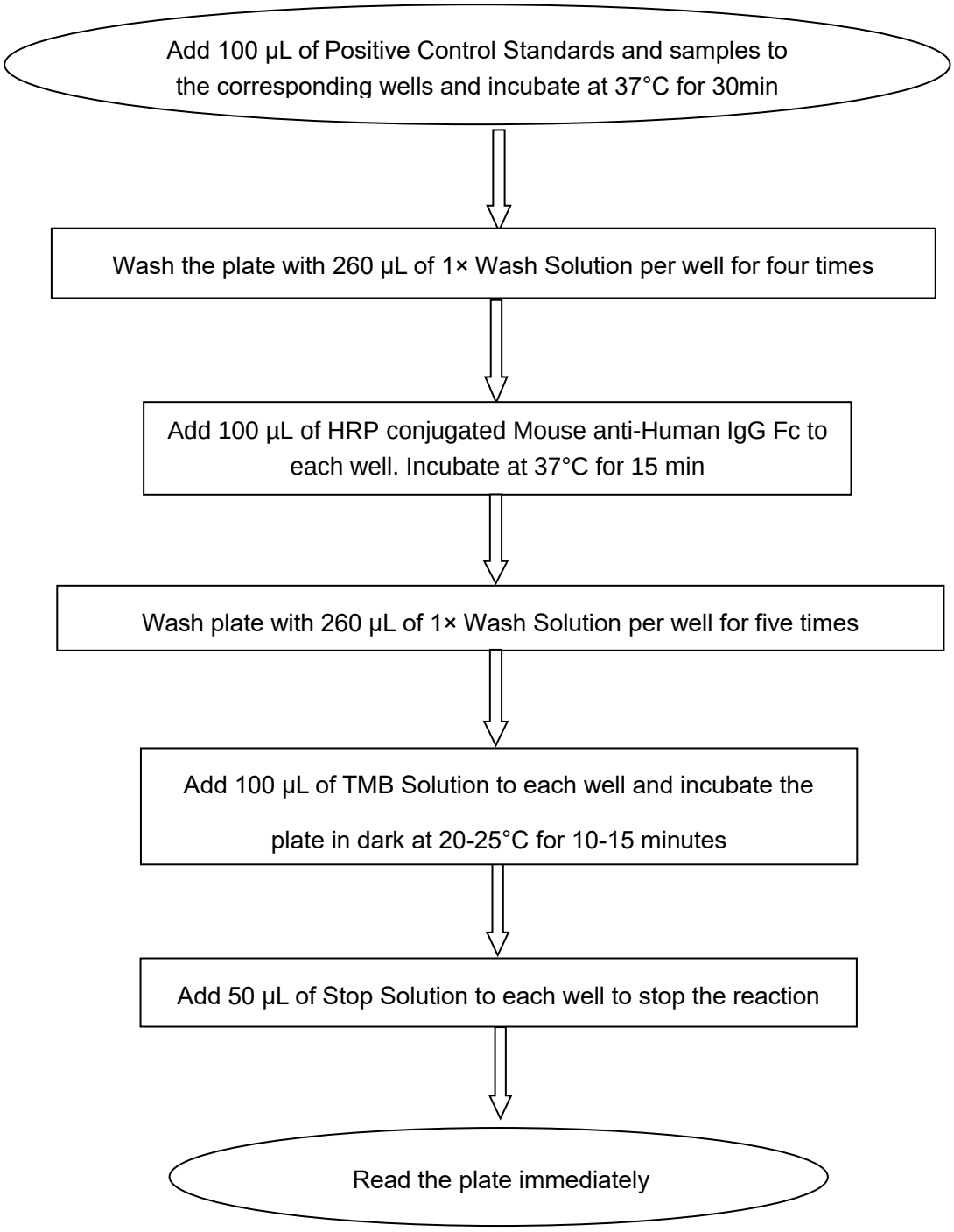
VII. ASSAY PROCEDUR SUMMARY



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VIII. TYPICAL ASSAY DATA

The standard curve, below is provided for demonstration only. The positive control standards must be re-tested for each test.

IgG Positive Control	OD450		
(μg/mL)	Duplicate 1	Duplicate 2	Average
25.000	2.0280	1.9441	1.9861
5.000	1.8307	1.7709	1.8008
1.000	1.7439	1.7703	1.7571
0.200	1.4388	1.4067	1.4228
0.040	0.6665	0.6641	0.6653
0.008	0.1656	0.1703	0.1680
0.000	0.0531	0.0518	0.0525

IX. PRECISION

Intra-assay: Three different known levels of control were spiked into sample buffer as test samples. All samples were tested 10 times on the same plate to evaluate intra-assay precision of the kit. Intra-assay precision of this kit is less than or equal to 10%.

Inter-assay: Three different known levels of control were spiked into sample buffer as test samples. All samples were tested in 6 separate assays to evaluate intra-assay precision of the kit. Inter-assay precision of this kit is less than or equal to 15%.

X. Rules for IgG evaluation

The IgG positive control of 0.008 μg/mL is a weak positive standard, and 1 μg/mL IgG positive control is a strong positive standard.

When the OD value of the sample is less than the OD value of 0.008 μg/mL standard, the SARS CoV S1-RBD IgG in the sample is inferred to be negative;

When the OD value of the sample is greater than or equal to the OD value of 0.008 μg/mL standard, the SARS CoV S1-RBD IgG in the sample is inferred to be positive;

When the OD value of the sample is greater than or equal to the OD value of 1 μg/mL standard, the SARS CoV S1-RBD IgG in the sample is inferred to be strongly positive.

The result is not used as a clinical diagnosis basis.

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XI. 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
Poor Standard Curve	Improper preparation of positive control	Prepare new positive controls as the manual describes
	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Pipetting error	Check pipette calibration and repeat assay
	Components are used from other lots or sources	Never substitute any components from another kit
	Components are not brought to room temperature prior to assay	Repeat assay with components that have been equilibrated to room temperature
	Incubation steps are performed at wrong temperatures	Perform incubation step as the manual describes
Weak/No Signal	Substrate are not added or added at the wrong time	Follow the manual to add the substrate properly

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	Components are used from other lots or sources	Use only lot-specific components
	Substrate are 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 immediately	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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