SARS-CoV-2 S1RBD IgG ELISA Kit
(Cat No. 41A223)

For the qualitative determination of human anti-SARS-CoV-2 spike protein S1 receptor-binding domain (S1RBD) ELISA (IgG class antibodies) in human serum or plasma samples

This package insert must be read in its entirety before using this product

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INTENDED USE

SARS-CoV-2 S1RBD IgG ELISA kit is a highly sensitive and specific enzyme-linked immunosorbent assay (ELISA) for the detection and qualitative measurement of IgG class antibodies against the spike protein S1 receptor-binding domain (S1RBD) of SARS-CoV-2 virus in human blood.

This product is intended for use by professional persons only.

SUMMARY

In December 2019, a novel coronavirus, now officially named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been identified in Wuhan China, which caused the outbreak of a coronavirus-associated acute respiratory disease called coronavirus disease 19 (COVID-19). Signs and symptoms of COVID-19 may occur 2 to 14 days after infection, which include fever, cough, shortness of breath or difficulties in breathing, pain in the muscle and tiredness. In severe cases, the infection can further lead to pneumonia, severe acute respiratory syndrome (SARS), kidney failure and death.

The spike protein (S) is an envelope-anchored protein that mediates the recognition and binding of SARS-CoV-2 to host cells. S can be further cleaved by the host protease into two subunits called S1 and S2. S1 polypeptide contain a receptor binding domain (S1RBD) crucial for the specific recognition and interaction with the human receptor ACE2, which is the first and the most essential step for the virus infection.

ASSAY PRINCIPLE

ImmunoDiagnostics SARS-CoV-2 S1RBD IgG ELISA kit is a two-step incubation immunoassay kit. Recombinant spike protein S1 receptor-binding domain (S1RBD) of SARS-CoV-2 pre-coated onto the polystyrene microwell strips can specifically recognize anti-S1RBD antibodies in human serum or plasma specimen. After a 1-hour incubation, anti-S1RBD antibodies are captured by immobilized S1RBD protein while the unbound components are washed away. Afterwards, a detection solution containing HRP-conjugated anti-human IgG is added for another 1-hour incubation, wherein HRP-conjugated anti-human IgG binds to the IgG class antibodies previously bound to S1RBD protein on the plate. After removal of nonspecific bindings, a HRP substrate solution containing 3,3′,5,5′-Tetramethylbenzidine (TMB) is added, resulting in the formation of a blue color. Color reaction is stopped by 2M H_2SO_4, transforming the blue color to yellow signals, which is quantified by an absorbance microplate reader at 450nm. The color intensity is proportional to the amount of anti- S1RBP antibodies captured inside the wells.

SUPPLIED REAGENTS AND MATERIALS

A	SARS-CoV-2 S1RBD coated ELISA plate	12 strips of 8 wells (96 wells in total) in a white strip holder and sealed in a foil bag with desiccant. Each well contains recombinant S1RBD of SARS-CoV-2. The microwell strips can be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with

		the desiccant and return to 2-8°C. Once opened, stable for 4 weeks at 2-8°C.
B	5x Assay Buffer	1 x 20 ml
C	10x Wash Buffer	1 x 40 ml
D	100x Detection Antibody Solution	1 x 0.12 ml
E	Substrate Solution	1 x 12 ml
F	Stop Solution	1 x 12 ml
G	Blank Control	2 x 1 ml

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipettes and pipette tips.
- 96-well plate or manual strip washer.
- Buffer and reagent reservoirs.
- Paper towels or absorbent paper.
- A microplate reader capable of reading absorbency at 450 nm or dual wavelength at 450/600~650nm.
- Distilled water or deionized water.

STORAGE AND PREPARATION OF TEST SAMPLES

- Test samples are suggested to be assayed immediately after separation of serum or plasma, or preferably stored frozen (-20^{0}C or below) in aliquots. Multiple freeze-thaw cycles should be avoided. Duplicate test is recommended.
- Serum or plasma specimens with EDTA, sodium citrate or heparin can be tested. Highly lipaemic, icteric, or hemolytic specimens are not recommended. Specimens with visible microbial contamination should not be used.
- When required, vortex test serum or plasma samples at room temperature to ensure homogeneity. Then centrifuge samples at 10,000 to 15,000 rpm for 5 minutes prior to assay to remove particulates. Please do not omit this centrifugation step if samples are cloudy and containing particles.

STORAGE AND STABILITY

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antigen-coated strips from the microplate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month. To assure maximum performance, protect the reagents from contamination with microorganism or chemicals during storage.

PREPARATION OF REAGENTS SUPPLIED

1. 1×Assay buffer.

Prepare 1x assay buffer by mixing the 5x assay buffer (30 ml) with 120 ml of distilled water or deionized water. If precipitates are observed in the 5x assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1x assay buffer may be stored at 2- 8°C for up to one month.

2. 1xWash buffer

Prepare 1xWash buffer by mixing the 10xWash buffer (40ml) with 360ml of distilled water or deionized water. If precipitates are observed in the 10xWash buffer bottle, warm the bottle in a 37°C water batch until the precipitates disappear. The 1xWash buffer may be stored at 2-8°C for up to one month.

3. 1x Detection antibody solution

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µl of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

SAMPLE PREPARATION

Serum or plasma sample is generally required a 100-fold dilution in the 1×Assay buffer. A suggested dilution step is to add 10 µl of sample to 990 µl of 1×Assay buffer. Dilution factor can be adjusted based on the titre of the antibodies in the samples

ASSAY PROCEDURES

Please equilibrate all the reagents to room temperature (20-25°C) for at least 30 minutes before use.

Step 1	**Adding controls and specimen:** Add 100µl of Specimen and 100µl of Blank Control into their respective wells. Duplicate test is recommended for both Specimen and Blank. Note: Use a separate disposal pipette tip for each Specimen and Blank to avoid cross-contamination. Mix by tapping the plate gently.
Step 2	**Incubation:** Cover the plate and incubate at room temperature for 1 hour.
Step 3	**Washing:** Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µl of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.

Step 4	**Adding HRP-conjugated Detection Solution:** Add 100 µl of 1×Detection Solution to each well.
Step 5	**Incubation:** Cover the plate and incubate at room temperature for 1 hour.
Step 6	**Washing:** Wash each well 4 times as described in step 3.
Step 7	**Colouring:** Add 100 µl of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light**.
Step 8	**Stopping Reaction:** Add 100 µl of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
Step 9	**Measurement:** Measure absorbance of each well at 450 nm immediately. If a dual filter instrument is used, set the reference wavelength at 600~650nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 10 minutes after stopping the reaction).

QUALITY CONTROL

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. If the reading is based on single filter plate reader (450nm), all results should be calculated by subtracting the Blank well absorbance value.

The test results are valid if the Quality Control criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient specimen being analyzed.

- The Absorbance value of the Blank well should be < 0.100 at 450nm.

TYPICAL RESULTS

Samples	OD450
Blank Control	0.063
	0.052
Serum from healthy subjects	0.144
	0.158
	0.174
Serum from COVID-19 patients	0.511
	0.656
	0.361

- Clinical validation study of ImmunoDiagnostics SARS-CoV-2 S1RBD IgG ELISA was conducted in 2020 in Shenzhen, China. Samples were collected from COVID-19 confirmed cases with clinical

symptoms, laboratory abnormalities or pulmonary imaging manifestations. No tests have been performed on specimens from latent infections or patients in the incubation period.

- It is highly recommended that each laboratory should establish its own normal and pathological reference range for anti-S1RBD IgG level. Furthermore, it is also recommended that each laboratory should include its own panel of control samples in the assay.

PERFORMANCE CHARACTERISTICS

Inter Assay Precision	
Samples	**CV**
1	**7.66%**
2	**6.68%**
3	**5.13%**
Intra Assay Precision	
Samples	**CV**
1	**5.78%**
2	**6.31%**
3	**4.55%**

PRECAUTIONS AND SAFETY

The ELISA assays are time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps.

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.

2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.

3. CAUTION - CRITICAL STEP: Allow the reagents and specimens to reach room temperature (20-25°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.

4. Use only sufficient volume of specimen as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.

5. Do not touch the exterior bottom of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.

6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.

7. Avoid long time interruptions of assay steps. Assure same working conditions for all wells.

8. Calibrate the pipette frequently to assure the accuracy of specimens/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.

9. When adding specimens, do not touch the well's bottom with the pipette tip.

10. When measuring with a plate reader, determine the absorbance at 450nm or at 450/600~650nm.

11. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.

12. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.

13. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.

14. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.

15. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.

16. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: the Stop solution, the Substrate solution, and the Wash buffer.

17. The Stop solution 2M H_2SO_4 is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.

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SUMMARY OF ASSAY PROCEDURE

Add 100 µl of sample to each well.

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Incubate at room temperature for 1 hour.

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Aspirate and wash each well three times.

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Add 100 µl of 1×Detection antibody solution to each well.

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Incubate at room temperature for 1 hour.

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Aspirate and wash each well four times.

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Add 100 µl of Substrate solution to each well.

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Incubate at room temperature for 15 minutes.

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Add 100 µl of Stop solution to each well.

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Measure absorbance of each well at 450 nm.

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Calculation and Interpretation

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