

High-sensitive SARS-CoV-2 S1RBD IgG ELISA Kit (CAT NO: 41A240R)

For quantitative determination of human anti-SARS-CoV-2 S1RBD antibodies (IgG class) in human serum, plasma or other specimen

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

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BACKGROUND

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.

INTENDED USE

SARS-CoV-2 S1RBD IgG ELISA Kit is a highly sensitive and specific immunoassay developed by ImmunoDiagnostics for the precision detection and quantitative measurement of IgG class antibodies against the S1RBD of SARS-CoV-2 virus in human blood samples.

This product is intended for used by professional persons only.

This product is intended for research use only.

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 H₂SO₄, 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-S1RBD antibodies captured inside the wells. The unknown sample concentration can be interpolated from a standard curve generated by the humanized anti-S1RBD monoclonal antibody (mAb).

REAGENTS SUPPLIED

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.
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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	10 × Standard Solution	1 x 0.06 ml, 24 IU/ml (Humanized anti-S1RBD monoclonal antibody, calibrated with WHO International standard (NIBSC code: 20/136) and expressed as International Unit (IU))

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips.
2. Beakers, flasks, cylinders necessary for preparation of reagents.
3. Buffer and reagent reservoirs.
4. Paper towels or absorbent paper.
5. Plate reader capable of reading absorbency at 450 nm.
6. Distilled water or deionized water.
7. Statistical calculator with program to perform regression analysis.

STORAGE

- The kit should be stored at 2-8°C, and all reagents should be equilibrated to room temperature before use. Immediately after use remaining reagents should be returned to cold storage (2- 8°C).
- Expiry of the kit and reagents is stand on labels.
- Once opened, the strips may be stored at 2-8°C for up to one month.

SAMPLE COLLECTION AND STORAGE INSTRUCTIONS

Handle serum or plasma sample in accordance with National Committee for Clinical Laboratory Standards guidelines for preventing transmission of blood-borne infection.

- Do not use grossly hemolyzed or lipemic samples.
- Human Serum: Use a blood separator tube and allow sample to clot for 30 minutes, then centrifuge for 10 minutes at 1000g.

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. **When the human plasma is tested, it should be diluted 100-fold at least.**

- Samples cannot be tested immediately should be aliquoted and must be stored frozen below - 20°C. Avoid repeated freeze -thaw cycles.
- Perform preliminary experiment to determine the optimum detection sample dilution.

PRECAUTIONS FOR USE

- All chemicals should be considered as potentially hazardous. Avoid contact with skin and eyes. In the case of contact with skin or eyes wash with water.
- Do not use kit reagents beyond expiration date.
- Do not expose kit reagents to strong light.
- Do not pipet by mouth.
- Do not eat or smoke in area where kit reagents or samples are handled.
- Use only sufficient volume of specimen as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.

- Avoid contact of substrate solution with oxidizing agents and metal.
- Use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- 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.
- 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.
- Substrate solution must be at room temperature prior to use.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before use

1. 1×Wash buffer.

Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 ml) with 360 ml of distilled water or deionized water. **CAUTION!!!** If

precipitates are observed in the 10xWash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear.

Incomplete dissolution will lead to high background. The 1×Wash buffer may be stored at 2-8°C for up to one month.

2. 1×Assay buffer.

Prepare 1x assay buffer by mixing the 5x assay buffer (20 ml) with 80 ml of distilled water or deionized water. **CAUTION!!!** If

precipitates are observed in the 5x assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear.

Incomplete dissolution will lead to high background. The 1x assay buffer may be stored at 2- 8°C for up to one month.

3. 1×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 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 pipetted.

PREPARATION OF STANDARD AND SAMPLES

Standard preparation:

Centrifuge 10x standard solution tube briefly before opening the cap.

Add 540 μL 1 \times assay buffer into the tube to generate the first standard (2.4 IU/ml). Prepare serially diluted standards using 1 \times assay buffer as follow:

	Standard Volume	Volume of 1 \times assay buffer	Concentration (IU/mL)
1	2.4 IU/mL	-	2.4
2	300 μL of 2.4 IU/mL	300 μL	1.2
3	300 μL of 1.2 IU/mL	300 μL	0.6
4	300 μL of 0.6 IU/mL	300 μL	0.3
5	300 μL of 0.3 IU/mL	300 μL	0.15

1x Assay buffer serves as the blank (0 IU/ml).

Note: The reconstituted standard stock should be aliquoted and stored at 2-8 $^{\circ}\text{C}$ for up to one month.

Sample preparation:

Serum or plasma sample is generally required a **100-fold dilution** in the 1X Assay buffer. A suggested dilution step is to add 2 μL of sample to 198 μL of 1X Assay buffer. Dilution factor can be adjusted based on the titre of the antibodies in the samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 100 μ l of standards or samples per well, and incubate at room temperature for 1 hour, with shaking at 600 rpm. (Note: incubation without shaking proportionally decreases the signal by approximately 30% If no shaker available, prolong the incubation time to 2 hours).
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 μ l of 1 \times Wash buffer to each well and incubate for 1 minute. Discard the 1 \times Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total of 4 times.
3. Add 100 μ l of 1 \times Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 4 times as described in step 2.
5. Add 100 μ l of Substrate solution to each well, incubate at room temperature for 15 minutes. Protect from light.
6. Add 100 μ l of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
7. Determine the optical density of each well at 450 nm immediately.

CACULATION

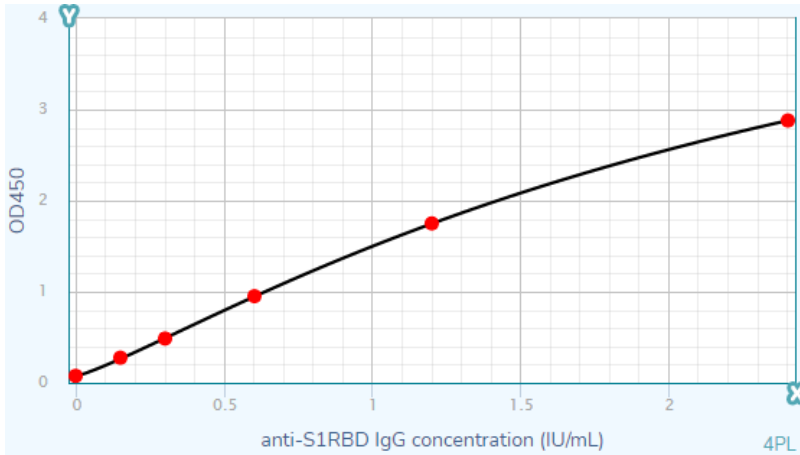
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against Human anti-S1 mAb (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. It is recommended to use the 4-parameter curve fitting for calculation.
3. Determine human anti-S1 IgG concentration from standard curve and then multiply the dilution factor to get the final concentration of the human anti-S1 IgG in samples.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each assay.

(IU/ml)	Absorbance (450 nm)	Blanked Absorbance
0	0.076	0
0.15	0.276	0.2
0.3	0.486	0.41
0.6	0.951	0.875
1.2	1.75	1.674
2.4	2.88	2.804

Anti-S1RBD IgG Standard Curve



RECOMMENDED INTERPRETATIONS OF THE RESULTS

Negative Result:

-The test result is deemed to be negative when the final concentration of the anti-S1RBD IgG in a sample is less than 5 IU/mL.

Negative result indicates that no SARS-CoV-2 S1RBD IgG have been detected with ImmunoDiagnostics SARS-CoV-2 S1RBD IgG ELISA.

Positive Result:

- Positive results: The test result is deemed to be positive when the final concentration of the anti-S1RBD IgG in a sample is equal to or more than 5 IU/mL. Positive result indicates the presence of SARS-CoV-2 S1RBD IgG antibodies in a sample.

PRECISION

Intra-assay: Three different known levels of positive control were spiked into sample buffer as test samples. All samples were tested on

the same plate to evaluate intra-assay precision of the kit. The Intra-assay precision of this kit is less than 5%.

Inter-assay: Three different known levels of control were spiked into sample buffer as test samples. All samples were tested in 3 separate assays to evaluate intra-assay precision of the kit. Inter-assay precision of this kit is less than 7%.

TRACEABILITY:

The WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/136) has been tested by the S1RBD IgG ELISA kit.

Sample	Theoretical Value (IU/mL)	Test 1 (IU/mL)	Test 2 (IU/mL)	Test 3 (IU/mL)	AVG (IU/mL)	Recovery
WHO STD (1/100)	10 IU/mL	OVER	OVER	OVER	/	/
WHO STD (1/200)	5 IU/mL	OVER	OVER	OVER	/	/
WHO STD (1/500)	2 IU/mL	2.12	2.09	2.09	2.10	99.1%
WHO STD (1/1000)	1 IU/mL	1.02	1.02	1.03	1.02	101.7%
WHO STD (1/2500)	0.2IU/mL	0.2	0.21	0.2	0.20	102.8%
WHO STD (1/12500)	0.04IU/mL	0.036	0.031	0.03	0.03	82.5%

SENSITIVITY AND SPECIFICITY

We have tested this assay in blood samples of 60 well-characterized COVID-19 subjects, and 58 of 60 subjects were anti-SARS-CoV-2 S1RBD IgG positive. 100 blood samples from healthy individuals were tested, and none of them were positive. **The sensitivity of this assay is $\geq 96\%$, and the specificity of this assay is $\geq 98\%$.**

SUMMARY OF ASSAY PROCEDURE

Add 100 μ l of standard/sample to each well.



Incubate at room temperature for 1 hour with shaking at 600 rpm. (If shaker is not available, prolong the incubation time to 2 hours.)



Aspirate and wash each well three times.



Add 100 μ l of 1 \times Detection antibody solution to each well.



Incubate at room temperature for 1 hour.



Aspirate and wash each well four times.



Add 100 μ l of Substrate solution to eachwell.



Incubate at room temperature for 15 minutes.



Add 100 μ l of Stop solution to each well.



Measure absorbance of each well at 450 nm.



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