

SARS-CoV-2 S1 IgG ELISA Kit (Cat No. 41A234)

(96 tests / box)

For the quantitative determination of human anti-SARS-CoV-2 spike protein S1 antibodies (IgG class) in human serum or plasma samples
(CE Marking No.: NL-CA002-2020-51026)

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

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INTENDED USE

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

This product is intended for the diagnosis of coronavirus disease 2019 (COVID-19).

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 comprises two functional subunits responsible for binding to the host cell receptor (S1 subunit) and fusion of the viral and cellular membranes (S2 subunit). The distal S1 subunit comprises the receptor-binding domain(s) and contributes to stabilization of the prefusion state of the membrane-anchored S2 subunit that contains the fusion machinery.

ASSAY PRINCIPLE

ImmunoDiagnostics SARS-CoV-2 S1 IgG ELISA kit is a two-step incubation immunoassay kit. Recombinant spike protein S1 of SARS-CoV-2 pre-coated onto the polystyrene microwell strips can specifically recognize anti-S1 antibodies in human serum or plasma specimen. After a 1-hour incubation, anti-S1 antibodies are captured by immobilized S1 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 S1 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-S1RBP antibodies captured inside the wells.

SUPPLIED REAGENTS AND MATERIALS

A	SARS-CoV-2 S1 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 S1 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	5× Assay Buffer	1 x 20 ml
C	10× Wash Buffer	1 x 40 ml

D	100× Detection Antibody Solution	1 × 0.12 ml
E	Substrate Solution	1 × 12 ml
F	Stop Solution	1 × 12 ml
G	10 × Standard Solution	1 × 0.06 ml, 27.2 IU/ml (Humanized anti-S1RBD monoclonal antibody, calibrated with WHO International standard (NIBSC code: 20/136) and expressed as International Unit (IU))

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.
- Distilled water or deionized water.
- A Horizontal microplate shaker capable of 600rpm (optional).

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°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 is stable until the expiry date only when stored at 2-8°C in sealed foil pouches. The expiry date is the last day of the month stated on the foil pouch and kit container.
- 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.
- Store the cut-off and positive control at 4°C, up to 3 months.

PRECAUTIONS AND SAFETY

- The ELISA assays are time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.
- 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.
- 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.

- 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.
- Use only sufficient volume of specimen as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.
- 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.
- 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.
- Avoid long time interruptions of assay steps. Assure same working conditions for all wells.
- 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.
- When adding specimens, do not touch the well's bottom with the pipette tip.
- When measuring with a plate reader, determine the absorbance at 450nm.
- 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.
- All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.
- Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
- Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
- 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.
- 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.
- The Stop solution 2M H₂SO₄ 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.
- ProClinTM 300 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

PREPARATION OF REAGENTS SUPPLIED

1. 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.

2. 1xWash buffer

Prepare 1xWash buffer by mixing the 10xWash buffer (40ml) with 360ml 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 1xWash buffer may be stored at 2-8°C for up to one month.

3. 1x Detection antibody solution

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

4. Preparation of Standard

Centrifuge the 10 × standard solution tube briefly before opening the cap. Add 450 µL 1 × assay buffer into the tube to generate the first standard (2.72 IU/mL). Prepare serially diluted standards using 1 × assay buffer as follow:

	Standard Volume	Volume of 1 × assay buffer	Concentration
1	2.72IU/mL	-	2.72 IU/mL
2	250 µL of 2.72IU/mL	250 µL	1.36 IU/mL
3	250 µL of 1.36IU/mL	250 µL	0.68 IU/mL
4	250 µL of 0.68 IU/mL	250 µL	0.34 IU/mL
5	250 µL of 0.34 IU/mL	250 µL	0.17 IU/mL

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

Note: The reconstituted standard stock should be aliquoted and stored at 2-8 °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 titer 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	<p>Adding controls and specimen: Add 100 µL of Specimen or Standard into their respective wells. Duplicate test is recommended. Note: Use a separate disposal pipette tip for each test to avoid cross-contamination. Mix by tapping the plate gently.</p>
Step 2	<p>Incubation: Cover the plate and incubate at room temperature for 1 hour, preferably with shaking at 600rpm if a shaker is available.</p>

Step 3	Washing: 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 3 washes.
Step 4	Adding HRP-conjugated Detection Solution: Add 100 μ L of 1 \times 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 μ L of Substrate solution to each well, incubate at room temperature for 15 minutes. Protect from light.
Step 8	Stopping Reaction: Add 100 μ L 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. 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.

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.

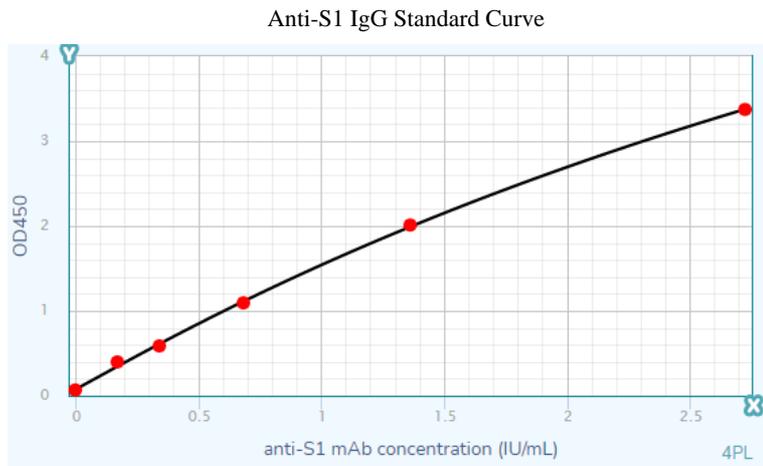
The Absorbance value of the blank (1 \times Assay buffer) should be < 0.150 at 450nm.

The Absorbance value of 0.68 IU/mL Standard should be > 0.500 at 450nm.

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	0.068	0
0.17	0.398	0.33
0.34	0.587	0.519
0.68	1.094	1.026
1.36	2.011	1.943
2.72	3.373	3.305



RECOMMENDED INTERPRETATIONS OF THE RESULTS

Negative Result:

The test result is deemed to be negative when the final concentration of the anti-S1 IgG in a sample is less than 5 IU/mL.

Negative result indicates that no SARS-CoV-2 S1 IgG have been detected with ImmunoDiagnostics SARS-CoV-2 S1 IgG ELISA.

Positive Result:

Positive results: The test result is deemed to be positive when the final concentration of the anti-S1 IgG in a sample is equal to or more than 5 IU/mL. Positive result indicates that the presents of SARS-CoV-2 S1 IgG antibodies in a sample.

Clinical validation study of ImmunoDiagnostics SARS-CoV-2 S1 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. The kit showed higher positive detection rate in specimens from patients with delayed onset. Therefore, the interpretation of the test results should consider the specimen's collection time.

It is highly recommended that each laboratory should establish its own normal and pathological reference range for anti-S1 IgG level. Furthermore, it is also recommended that each laboratory should include its own panel of control samples in the assay.

TRACEABILITY:

The WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code: 20/136) has been tested by the S1 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	2.031	2.039	2.040	2.037	101.8%
WHO STD (1/1000)	1 IU/mL	1.013	1.021	1.018	1.017	101.7%
WHO STD (1/2500)	0.2IU/mL	0.204	0.211	0.202	0.206	102.8%
WHO STD (1/12500)	0.04IU/mL	0.036	0.032	0.031	0.033	82.5%

PERFORMANCE CHARACTERISTICS

Sensitivity	≥ 97% (n=80)
Specificity	≥99% (n=314)
Inter Assay Precision	
Samples	CV
1	3.29%
2	3.63%
3	5.52%
Intra Assay Precision	
Samples	CV
1	8.16%
2	9.01%
3	7.84%

LIMITATIONS

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immune-suppressed individuals. Therefore, negative results obtained with ImmunoDiagnostics SARS-CoV-2 S1 IgG ELISA are only indication that the specimen does not contain detectable level of antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with the virus.
3. False positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step. For more information regarding ImmunoDiagnostics ELISA Troubleshooting, please contact ImmunoDiagnostics technical support for further assistance.
4. The most common assay mistakes are using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
5. The prevalence of the marker will affect the assay's predictive values.
6. This assay cannot be utilized to test pooled (mixed) serum or plasma. The kit has been evaluated only with individual serum or plasma specimens.

SYMBOLS

	Manufacturer		EC Declaration of Conformity
	Expiry date		Consult Instruction
	Lot number		Store
	Catalog number		Caution

IVD	<i>In Vitro</i> Diagnostic Device		Bio Hazard
CONTROL -	Negative control		

SAFETY CONSIDERATIONS

Streptavidin Peroxidase (SA-POD)

Signal word: Warning

Hazard statement(s)



H317: May cause an allergic skin reaction Precautionary statement(s)

P280: Wear protective gloves/protective clothing/ eye protection/face protection

P302 + P352: IF ON SKIN: Wash with plenty of soap and water

P333 + P313: If skin irritation or rash occurs: Get medical advice/attention

P362 + P364: Take off contaminated clothing and wash it before reuse

Peroxidase Substrate (TMB)

Signal word: Danger

Hazard statement(s)



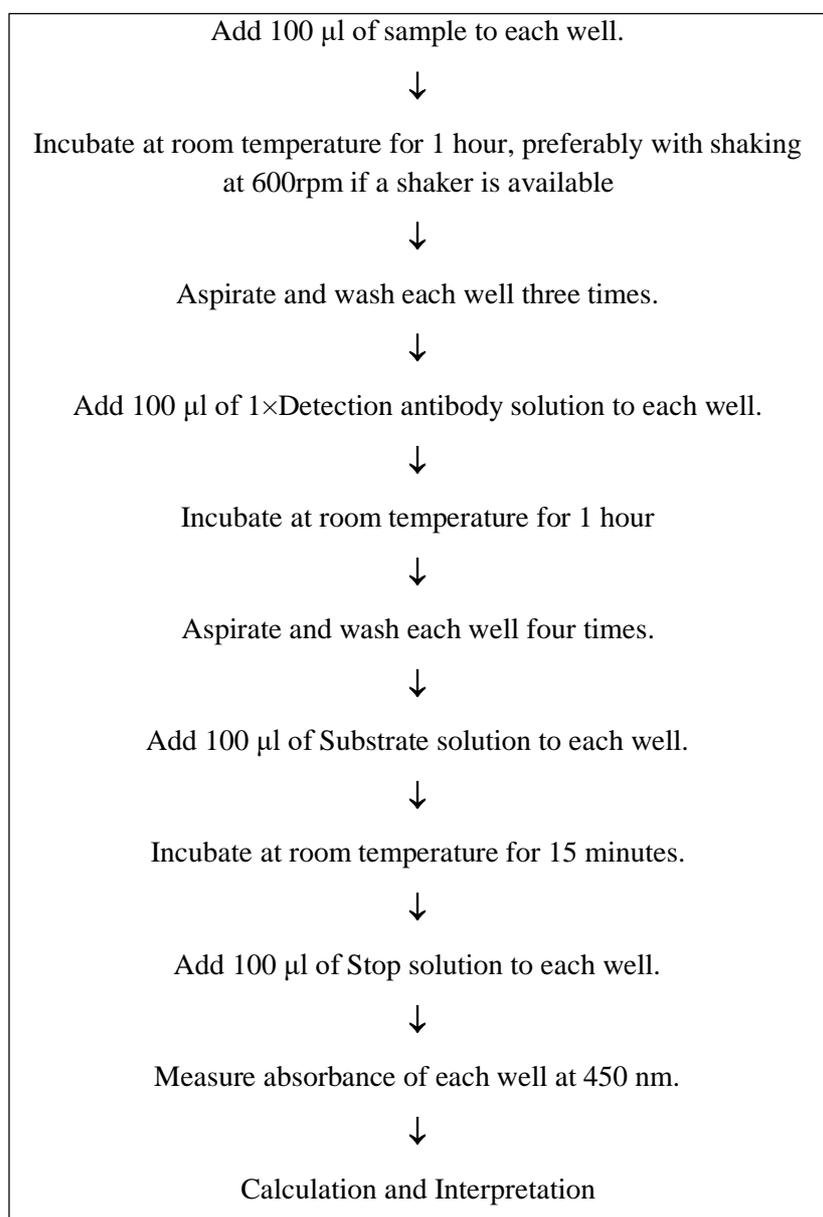
H360D: May damage the unborn child

Precautionary statement(s)

P280: Wear protective gloves/protective clothing/ eye protection/face protection

P308 + P313: If exposed or concerned: Get medical advice/attention

SUMMARY OF ASSAY PROCEDURE



➤ Basic Information

Registrant/manufacturer: ImmunoDiagnostics Limited

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