SunLong Biotech Co.,LTD

Tel: 0086-571-56623320 Fax:0086-571-56623318

E-mail:sales@sunlongbiotech.com

www.sunlongbiotech.com

Human SARS-CoV-2 Spike Protein S1 IgG (S1-IgG) ELISA Kit

48**Tests**

Catalogue Number:SL3219Hu

Store all reagents at -20℃

Validity Period: 12 months

For samples:

In serum, plasma, culture media or any biological fluid.

FOR RESEARCH USE ONLY！

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS！

PLEASE READTHROUGH ENTIRE PROCEDURE BEFORE BEGINNING !

Human SARS-CoV-2 Spike Protein S1 IgG (S1-IgG) ELISA Kit

FOR RESEARCH USE ONLY

Drug Names

Generic Name：Human SARS-CoV-2 Spike Protein S1 IgG (S1-IgG) ELISA Kit

Purpose

Our Human SARS-CoV-2 Spike Protein S1 IgG ELISA kit is to assay S1-IgG levels in Human serum, plasma, culture media or any biological fluid.

Principle

This kit employs direct ELISA Technique. Direct ELISA is based on Antigen-antibody response characteristics.It is suitable for in vitro semi-quantitative detection of human serum, plasma or cell culture supernatant and organizations in the natural SARS-CoV-2 Spike Protein S1 IgG concentration. Detection of other special sample please contact our technical support. The kit is for research use only. Please read the instructions carefully before using and check the kit components.

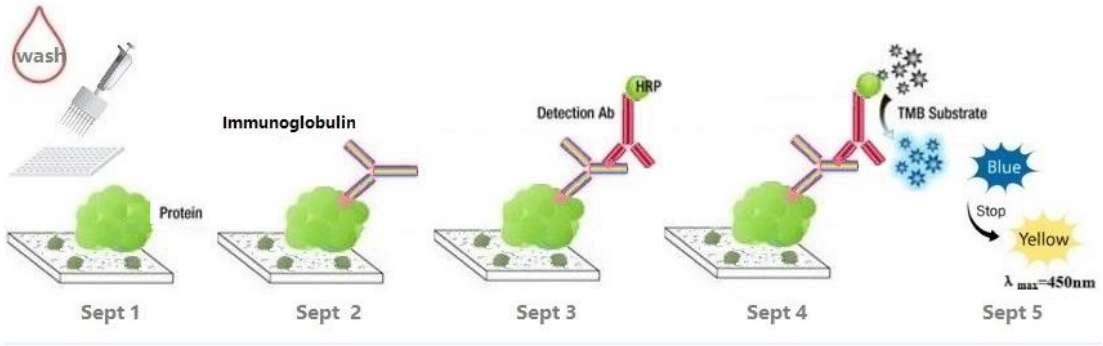
Procedure:

1. Ligation of specific antigens and solid phase carriers to form immobilized antigens. Wash unbound antigen and impurities. Seal the remaining binding sites with extraneous proteins.
2. Combine the test object with the immobilized antigen for contact reaction. After a period of time, the antibody and the antigen on the carrier are combined into an antigen-antibody complex. Wash unbound antibodies and impurities.
3. Add biotin-labeled antibodies to bind to antibodies on the immune complex. Wash unbound biotin-labeled antibodies thoroughly. The amount of enzyme on the carrier is positive related to the amount of the substance in the sample.
4. Add horseradish peroxidase-labeled avidin and combine it with biotin-labeled antibody. Thoroughly wash the incorporated enzyme label. The amount of enzyme on the carrier is positive related to the amount of the substance in the sample.
5. Add substrate for coloring and calculate sample concentration.Note: antibody molecule can be marked on several biotin molecules and biotin molecule can be connected with a HRP-Avidin to numbers of horseradish peroxidases combining with antibodies which shows higher sensitivity and amplification effect comparing with traditional direct HRP-Antibodies.

Detection principle of SARS-CoV-2 Spike Protein S1 IgG ELISA kit

This experiment use direct ELISA technique and the ELISA Kit provided is typical. The pre-coated antigen is Recombinant SARS-CoV-2 protein and the detecting antibody is polyclonal antibody with biotin labeled. Samples, standards, biotinylated secondary antibodies, and avidin-peroxidase conjugates were added to the ELISA plate wells in sequence, washed with PBS or TBS, and finally stained with TMB substrate. TMB turns into blue in peroxidase catalytic and finally turns into yellow under the action of acid. The color depth and the testing factors in samples are positively correlated.

Schematic diagram of the SARS-CoV-2 Spike Protein S1 IgG ELISA kits



Kit composition

Name	96 Tests	48 Tests	Storage
Precoated plate	8×12	8×6	4/-20℃
SARS-CoV-2 Spike Protein S1 IgG Standards	2 vial	1 vial	4/-20℃
Biotinylated antibody(1:100)	1vial	1 vial	4/-20℃
Enzyme conjugate(1:100)	1vial	1 vial	4/-20℃
Enzyme diluent	1vial	1 vial	4/-20℃
Antibody diluent	1vial	1 vial	4/-20℃
Standard diluent	1vial	1 vial	4/-20℃
Sample diluent	1vial	1 vial	4/-20℃
Washing buffer (1:25)	1vial	1 vial	4/-20℃
Colour Reagent A	1vial	1 vial	4/-20℃
Colour Reagent B	1vial	1 vial	4/-20℃
Colour Reagent C	1vial	1 vial	4/-20℃
Instruction	1 set	1 set	RT

Note：RT：Room temperature|Standard：Frozen dried|Colour Reagent A: Avoid light

Necessary for testing their own test facilities and equipment

1. Microplate reader (450nm detection wavelength filter, 570nm or 630nm correction wavelength filters).
2. Washer (adjustable amount of liquid injection to ensure that each well 350μl lotion without overflow).
3. Clean benches, biological safety cabinets, fume hoods.
4. High-precision single-channel dispenser (range 0.5-10μl-20μl, 20-200μl,

200-1000μl).
5. High-precision multi-channel plus liquid (8 or 12, the range of 50-300μl of).
6. 37℃ incubator.
7. Low temperature centrifuge.
8. Refrigerators (4℃, -20℃, -86℃).
9. Analytical balance.
10. Scissors, tweezers, pliers, and so on.
11. Swirl mixing device, low-frequency oscillator, and so on.

Necessary for testing their own testing supplies and reagents
1. Centrifuge tube (capacity of 1.5ml, 5ml, and so on).
2. Disposable tip (range of 0.5-10μl-20μl, 20-200μl, 200-1000μl).
3. Pure water or distilled water.
4. Coordinate paper.
5. Absorbent paper.
6. EDTA, sodium citrate, heparin

Sample collection Note
1. The tube for blood collection should be free of pyrogen and endotoxin
2. Hemolysis and hyperlipidemia specimens can not be used to extracted serum and plasma.
3. The samples should appear clear and transparent. And all the suspension should be removed through centrifugation.
4. If collected samples are not timely detected, they should be divided according to single usage amount and frozen reserved in refrigerator at -20-80℃, avoiding the repeated freeze-thaw.
5. According to the actual situation of the samples, make proper multiple dilutions (Pre-experiment is strongly recommended in order to confirm the dilution ratio)
6. Collect specimens and try to gain double dosage to avoid specimens shortage for repeated assays in case that failure in one-assay delays experimental process.
7. Do protective measures when collecting specimens (e.g. wearing gloves, respirator, respirator, etc.), aware of the potential risk in all specimens.
8. Specimen processing should be inside the biological safety cabinet. Ensure proper use of the biological safety cabinet.

Sample preparation

1. **Serum preparation**

After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again.

2. **Plasma preparation**

Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifugated for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifugated again.

3. **Urine samples**

Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.

4. **Cell samples**

If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X100/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again.

5. **Tissue samples**

Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80℃ for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4℃. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

Note

1. The kit should be kept at 2-8℃ before being used. Except the redissolved standard samples, other Ingredients must not be frozen.
2. For the concentrated biotinylated SARS-CoV-2 Spike Protein S1 IgG antibody, the concentrated enzyme-conjugates have small size. Bumping or potential inversion of the tubes during transportation may cause the liquid sticking to wall or cap. Thus, the tubes should be shaken manually or centrifuged for 1 min at 1000rpm to shake off the adherent liquid down to the tube bottom.
3. Concentrated washing buffer may crystallize a little. Use water bath to help the dissolution during diluting process. The crystals must be totally dissolved when

preparing washing buffer.

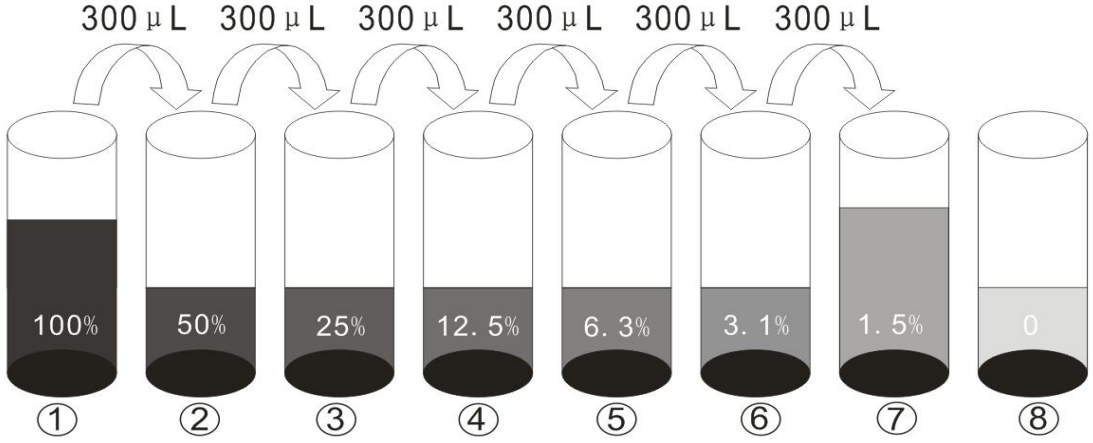
4. During testing process, the SARS-CoV-2 Spike Protein S1 IgG lyophilized standard sample shall be single-use and must not be divided. The sample will quickly inactivate after being dissolved because of its lower concentration.
5. Operation should be strictly in accordance with the instructions. Mixed usage of components with different batch number in this reagent is not allowed
6. Ensure the reagent well mixed by the spiral hybrid instrument. For the reagent in the microplate, adequate mixing is particularly important to test result. So it's better to employ the micro-oscillator (at the lowest frequency). If there is not micro-oscillator, shake the microplate manually for 1 min, slightly as like a circular movement to make sure reaction liquid in microplate well mixed
7. ELISA for experiment should be strictly operated according to manual standard and fully preheated beforehand.
8. During enzyme immunoassay, there should be multiple pores when testing SARS-CoV-2 Spike Protein S1 IgG standard samples
9. Put the unused microplates into raw foil bag at 2-8℃ for storage.
10. Chromogen reagent is sensitive to light. Therefore it should be free of being exposed to light.
11. Kits out of validity should not be used in experiment.
12. The determination of test results must be subject to ELISA's readings. When using dual-wavelength for test, the wavelength should be set at 450nm and 630nm respectively.
13. All the samples, washing liquid and wastes should be treated as biowaste. Colour Reagent C should be 1M sulfuric acid and pay attention to safety when it is used.
14. Sample-adding at every step should be taken by adding instrument. Calibrate accuracy of the adding device to avoid experiment error. The time of single sample-adding should be controlled within 5 min. Just in case of exceeding samples, the volley for sample-adding is proposed.
15. Adhesive closures do not reuse or according to the experiments need to be cropped. Stick a strip of adhesive to compaction
16. Test determination and standard curve should be made at same time in every experiment, so there better be multiple pores. If the content of test sample were too high (OD value of the sample is higher than that of sample well maximum concentration), dilute to certain multiple by sample diluents (n times), then test the result and multiply it by dilution ratios when making calculation.
17. The sample containing NaN3 can't be tested because NaN3 inhibit the activities of horseradish peroxidase (HRP).
18. When washing board by plate washer, the volume of liquid injection to each well should be more than 350μl. Check if the sampling head is jammed. Yet the water absorption material with Paper Scrap should be cautiously used while washing board manually, free of the reaction between exogenous peroxidase analogues and chromogen reagents.
19. After the reaction being terminated by Colour Reagent C, read OD within 10 min.
20. During multiple pores experiment, the calculation result shall be mean value.

21. Sample hemolysis may cause false positive result, so this test is not appropriate for sample hemolysis.
22. During test, the strips should be put into closed box after adding samples and the humidity around should be kept at about 60%
23. It is advisable to check the thermostat by frequent calibration to keep its inside temperature at 37℃. Ensure the experimental temperature being steady.
24. For 48T Elisa Kit, all components are 50% amount of 96T.
25. If there is any difference, the English instruction shall prevail.

Test preparation

1.Please get the Elisa Kit out of refrigerator 20 minutes in advance and take test when it balances to room temperature.
2. Dilute the concentrated washing solution with double distilled water (1:25). Put the unused back.
3. SARS-CoV-2 Spike Protein S1 IgG standard sample: Add Standard diluent 1.0ml into SARS-CoV-2 Spike Protein S1 IgG lyophilized standard sample and keep it still for 30 min. After the sample completely dissolved, mix it slightly and mark label on the tube①， then take dilution as needed.(It is recommended to using following concentration value to standard curve: 200, 100, 50, 25, 12.5, 6.25, 3.12U/ml). Note: Make sure the lyophilized standard completely dissolved and well mixed.
4. Legend of standard sample dilution method: Take 7 clean tubes and label them with ②,③,④,⑤,⑥,⑦,⑧ respectively. Add 300μl standard sample diluent into each tube. Pipette out 300μl diluent from tube ① to tube ② and mix well. Further Pipette out 300μl diluent from tube ② to tube ③, and mix well. Repeat steps above up to tube ⑦. Standard sample dilution in tube⑧is negative control.

Note: The redissolved standard liquid （ 200U/ml ） shall be discarded and not non-reusable.



Note: Reconstituted standard stock solution can not be reused.

5. Biotinylated SARS-CoV-2 Spike Protein S1 IgG antibody liquid: Referring to needed amount, employ antibody diluent to dilute the concentrated biotinylated antibody (1:100) to form biotinylated antibody liquid. The preparation should be done 30 min in advance. And it's only for use on that day

6. Enzyme-conjugate liquid: Referring to needed amount, dilute the concentrated enzyme-conjugate by enzyme-conjugate diluent (1:100) to form enzyme-conjugate liquid. The preparation should be done 30 min in advance. And it's only for use on that day.
7. Colour Reagent liquid: Prepare Colour Reagent liquid 30 min in advance with Colour Reagent A and Colour Reagent B by the proportion of 9:1.

Washing method

1.Automatic plate-washing machine: The required amount of lotion is 350μl and the injection and extraction interval should be 20 — 30secs. Be well aware of the operation instruction before putting the machine into practice.
2. Manual plate-washing machine: add 350μl lotion to each well and keep it still for 30secs. Shake individual wells as dry as you can and clean them with absorbent paper. During the plate-washing process, pay attention to the lotion-adding step to avoid contamination and well-jumping..

Steps

1.Take out needed strips from zip lock bag which balances to room temperature. The unused strips and desiccant should be put back into the sealed aluminum foil bag at 2-8℃ for storage.
2. Set aside blank wells (if dual-wavelength reading plate is used, the blank wells could be ignored)
3. Add samples or different concentration of SARS-CoV-2 Spike Protein S1 IgG standard samples to corresponding wells (100μl for each well), 0U/ml well should be filled with standard diluent. Seal the reaction wells with adhesive tapes, hatching in incubator at 37℃ for 90 min.
4. Prepare biotinylated SARS-CoV-2 Spike Protein S1 IgG antibody liquid 30min in advance.
5. Wash the Elisa plate 2 times
6. Add the biotinylated SARS-CoV-2 Spike Protein S1 IgG antibody liquid to each well (100μl for each). Seal reaction wells with adhesive tapes, hatching in incubator at 37℃ for 60 min.
7. Prepare enzyme-conjugate liquid 30min in advance.
8. Wash the Elisa plate 3 times
9. Add enzyme-conjugate liquid to each well except blank wells (100μl for each). Seal the reaction wells with adhesive tapes, hatching in incubator at 37℃ for 30 min.
10. Wash the Elisa plate 5 times.
11. Add 100μl Colour Reagent liquid to individual well (also into blank well), hatching in dark incubator at 37℃. When color for high concentration of standard curve become darker and color gradient appears, the hatching can be stopped. The chromogenic reaction should be controlled within 30 min.
12. Add 100μl Colour Reagent C to individual well (also into blank well). Mix well. Read OD（450nm）within 10 min.

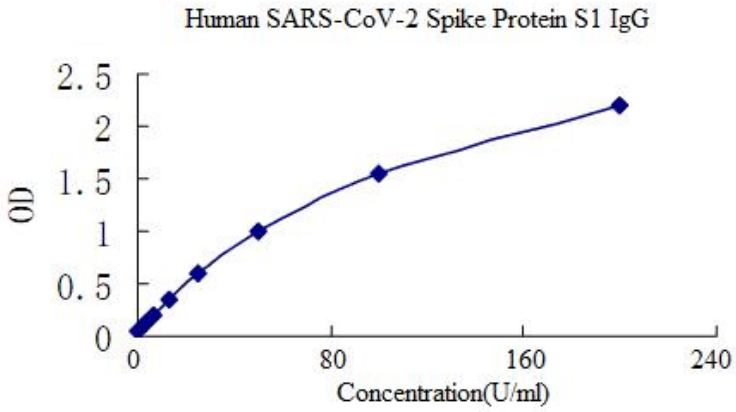
Note:It doesn't need to add any reagents in blank well except Color reagent solution and Color reagent C.

Result determination

1. OD value of each sample and specimen should minus that of blank well (if not, the standard curve of zero well should intersect at Y axis)
2. Draw standard curve manually. Take concentration value of samples as abscissa and OD readings as vertical coordinate. Use smooth line to connect each coordinate point of standard sample. The concentration of samples can be found by checking sample OD reading. It is recommended to employ the professional curve software (e.g. curve expert 1.3) to analyze and compute the result.
3. If the sample OD is higher than the upper limit of standard curve, the sample should be re-diluted and the experiment rerun. Multiply the result by dilution factor when calculating the unknown.

Note: This chart is only for reference. The calculation of specimens' content shall be subject to the standard curve made for samples in same experiment.

Reference curve



Note: This chart is for reference only, should be based on the test standard dwarfed standard curve to calculate the specimen content.

Summary of operating procedures

step	Summary of operating procedures
1	Prepare reagents, samples and standards.
2	Add the prepared samples and standard & incubate at 37 ℃ for 90 minutes.
3	Wash 2x, add Biotinylated Antibody solution & incubate at 37 ℃ for 60 minutes.
4	Wash 3x, then add the Enzyme working solution & incubate at 37 ℃ for 30 minutes.
5	Wash 5x, then add the Color Reagent solution & incubate at 37 ℃ up to 30 minutes.
6	Add the Color Reagent C.
7	Use microplate reader to measure OD within 10 minutes of adding Color

	Reagent C.
8	Calculate the content of samples being tested

Precision

Intra-Assay: CV<8%

Inter-Assay: CV<12%

Recovery70 - 110 percent.

Assay range

200 U/ml-3.12 U/ml

Sensitivity：

1.2 U/ml

Storage and validity

Storage-20℃ [Short-term should be placed 4℃(such as two weeks)]
Usesused in vitro quantitative analysis of liquid samples .
Production DateSee microtiter plates aluminum foil bag sealing stamp.
Validity12 months (-20℃).