SARS-CoV-2 Spike S1 Protein Antibody ELISA Kit

Catalog No.: RK04145

version: 2.0

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

Introduction

The kit applies for detecting the level of anti-SARS-CoV-2 Spike S1 Protein antibodies in serum and plasma.

Principle of the Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A Recombinant SARS-CoV-2 Spike S1 Protein has been pre-coated onto a microplate. Control Antibody and samples are pipetted into the wells and any SARS-CoV-2 Spike S1 Protein Antibody is bound by the immobilized Protein.Following incubation unbound Antibodies are removed during a wash step, and then a detection protein is added to the wells and binds to the combination of SARS-CoV-2 Spike S1 Protein Antibody- Control antibody in sample. Following a wash to remove any unbound combination, and an enzyme conjugate is added to the wells. Following incubation and wash steps, a substrate is added. A chromogenic reagent TMB is formed in proportion to the amount of SARS-CoV-2 Spike S1 Protein Antibody present in the sample. The reaction is terminated by acid and the absorbance is measured. A standard curve is prepared from

seven Control Antibody standard dilutions and SARS-CoV-2 Spike S1 Protein Antibody concentration determined.

Material Provided & Storage Conditions

Unopened kits can be stored at 2-8°C for 1 year, and opened products must be used within 1 month.

Part	Size	Cat. No.	Storage of opened/ reconstituted material
SARS-CoV-2 Spike S1 Protein Antigen Coated Plate	8×12	RM17529	Put the unused slats back in the aluminum foil bag with the desiccant and reseal them. They can be stored at 2-8°C for 1 month.
SARS-CoV-2 Spike S1 Protein Control Antibody（1000x）	1 ×40 μL	RM17530	It is not recommended to use again after redissolving.

SARS-CoV-2 Spike S1 Protein Concentrated Biotin Conjugate Antigen (250×)	1 ×40 μL	RM17531	Store at 2-8℃ for 1 month *
Streptavidin-HRP Concentrated (100 ×)	1 ×120 μL	RM17532	Store at 2-8℃ for 1 month *
Control/Sample Diluent (R1)	1 ×20mL	RM00023	Store at 2-8℃ for 1 month *
Biotin-Conjugate Antigen Diluent (R2)	1 ×12mL	RM00024	
Streptavidin-HRP Diluent(R3)	1 ×12mL	RM00025	
Wash Buffer(20x)	1 ×30mL	RM00026	
TMB Substrate	1 ×12mL	RM00027	
Stop Solution	1 ×6mL	RM00028	
Plate Sealers	4 Strips		
Specification	1		

***Note:** The specifications listed in the table are for 96T kit, and the amount of other components in the 48T kit are halved except for the standard, please be aware of this.

Other Supplies Required

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 570 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. Incubator.
6. Test tubes for dilution of standards and samples.

Precautions

***For Research use only，not be used for diagnosis.**

1. The kit should not be used beyond the expiration date on the kit label.
2. Do not mix or substitute reagents with those from other lots or other sources.
3. If the OD value of the sample obtained from the test exceeds the maximum detection limit of the product, please dilute the sample using the standard/sample diluent (R1) in the product. Therefore, it is recommended to pre-test the sample before formally testing the sample.
4. Sample addition, plate washing, incubation time, incubation temperature and other operations during the experiment will affect the final results, please strictly manage the experimental process and keep good records.
5. Variations in sample collection, processing, and storage may cause sample value differences.
6. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.
7. Reagents may be harmful, if ingested, rinse it with an

excess amount of tap water.

8. Stop Solution contains strong acid. Wear eye, hand, and face protection.
9. To ensure the best results, please refer to the labels or instructions for storage of relevant reagent components.
10. Mixing of the reagents after preparation is very important for the results, but some proteins or antibodies may be very sensitive to vigorous vortexing, which may cause loss of activity, so please use vortexing with caution.
11. Please use sterilised consumables for reagent preparation to avoid contamination of the reagents, which may affect the final test results.
12. In order to ensure the best detection effect, it is not recommended to reuse the working solution of the solubilised standard protein and related reagents after freezing.
13. The kit should be away from light when it is stored or incubated.
14. To avoid cross contamination, please use disposable pipette tips.
15. Please prepare all the kit components according to the Specification. If the kits will be used several times, please

seal the rest strips and preserve with desiccants. Do use up within 1 months.

16. The 48T kit is also suitable for the specification.

Sample Collection & Storage

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

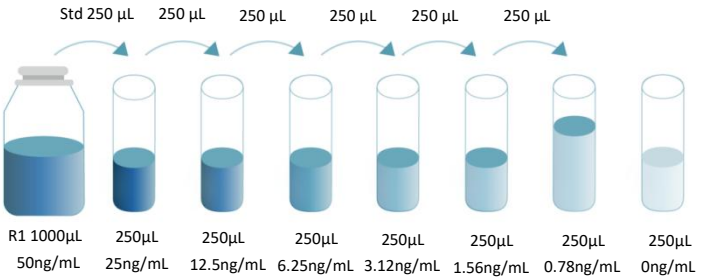
Plasma: Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles. (Note: Citrate plasma has not been validated for use in this assay.)

Note: It is suggested that all samples in a study be collected at the same time of the day. Avoid hemolytic and hyperlipidemia sample for serum and plasma.

Reagent Preparation

Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature, and mix gently until the crystals have completely dissolved.

Control Antibody - Dilute 1:1000 with the Control/Sample Diluent(R1) ,sit for a minimum of 15 minutes with gentle agitation prior to making dilutions (50ng/mL), Prepare EP tubes containing Control/Sample Diluent(R1), and produce a dilution series according to the picture shown below (recommended concentration for standard curve: 50, 25, 12.5, 6.25, 3.12, 1.56，0.78，0ng/mL).



Concentrated Biotin Conjugate Antigen (250x)- Dilute 1:250 with the Biotin-Conjugate Antigen Diluent (R2) before use, and

the diluted solution should be used within 30 min.

Working Streptavidin-HRP - Dilute 1:100 of Concentrated Streptavidin-HRP (100x) with Streptavidin-HRP Diluent (R3) before use. For example: Add 20 μ L of Concentrated Streptavidin-HRP (100x) to 1980 μ L Streptavidin-HRP Diluent (R3) to prepare 2000μL Working Streptavidin-HRP Buffer.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 1:20 with double distilled or deionized water before use. For example: Add 20 mL of Wash Buffer Concentrate to 380 mL of deionized or distilled water to prepare 400 mL of Wash Buffer.

Sample preparation

For different samples, the appropriate dilution level should be chosen on a case-by-case basis.

Serum/plasma:Due to individual differences, please anticipate the concentration range of the sample in advance and determine the dilution of the sample to be examined by pre-testing.Please refer to the following dilution instructions.

Dilution Method

For 100 fold dilution: One-step dilution. Add 5 μL sample to 495 μL sample diluent to yield 100 fold dilution.

For 1000 fold dilution: Two-step dilution. Add 5 μL sample to 95 μL sample diluent to yield 20 fold dilution, then add 5 μL 20 fold diluted sample to 245 μL sample diluent, after this, the neat sample has been diluted at 1000 fold successfully.

Each dilution step should be performed at a minimum of 3 μL and at a maximum of 100-fold dilution. Each dilution step should be mixed well to avoid foaming.

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working controls and samples as directed in the previous sections.Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal properly.
2. Add wash buffer 350 μL/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes.
3. Add 100 μL Control/sample Diluent (R1) in a blank well.
4. Add 100 μL different concentration of Control Antibody or samples in other wells. Cover with the adhesive sealer provided. Shake with Micro-oscillator (250r/min). Incubate for 2 hours at room temperature.Record the plate layout of standards and sample assay.
5. Prepare the Concentrated Biotin Conjugate Antigen (250x) Working Solution 15 minutes early before use.
6. Repeat the aspiration/wash as in step 2.

7. Add 100 μL Working Biotin Conjugate Antigen in each well, cover with new adhesive sealer provided.shake with Micro-oscillator (250 r/min). Incubate for 1 hours at room temperature.
8. Prepare the Streptavidin-HRP Concentrated (100x) Working Solution 15minutes early before use.
9. Repeat the aspiration/wash as in step 2.
10. Add 100 μL Working Streptavidin-HRP in each well, cover with new adhesive strip provided. shake with Micro-oscillator (250 r/min). Incubate for 0.5 hours at room temperature.
11. During the incubation, turn on the microplate reader to warm up for 30 minutes before measuring.
12. Repeat the aspiration/wash as in step 2.
13. Add 100 μL TMB Substrate to each well. Incubate for 15-20 minutes at room temperature. Protect from light.
14. Add 50 μL Stop Solution, determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for

optical imperfections in the plate. Readings made directly at 450 nm without correction may cause higher value and less accurate result.

Assay Procedure Summary

Prepare the standard and reagents

Wash 3 times

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Add 100 μL of Control or test samples to each well

Incubate for 2 hours at RT, then wash 3 times

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Add 100 μL Biotin-Conjugate Protein Working Solution

Incubate for 1 hours at RT, then wash 3 times

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Add 100 μL Working Streptavidin-HRP

Incubate for 0.5 hour at RT, then wash 3 times

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Add 100 μL Substrate Solution

Incubate for 15-20 min at RT under dark condition

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Add 50 μL Stop Solution

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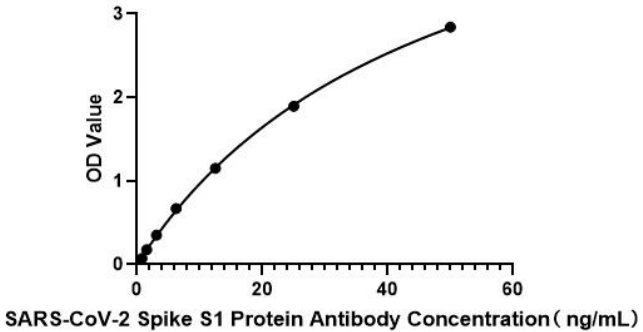
Detect the optical density within 5 minutes under 450nm.

Correction Wavelength set at 570nm or 630nm

Calculation of Results

1. Calculate the average OD value of the replicate wells for each concentration of standard protein, quality control, sample, etc. The OD value of each test should be subtracted from the OD value of the blank wells as well as the OD value of the sub-wavelength.
2. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the SARS-CoV-2 Spike S1 Protein Antibody concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted,the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data



The standard curves are provided for demonstration only. A standard curve should be generated for each set of SARS-CoV-2 Spike S1 Protein Antibody assayed.

Detection Range

0.78-50ng/mL

Sensitivity

The minimum detectable dose (MDD) of SARS-CoV-2 Spike S1 Protein Antibody typically less than 0.18 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Precision

Intra-plate Precision

3 samples with low, middle and high level SARS-CoV-2 Spike S1 Protein Antibody were tested 20 times on one plate, respectively.

Intra-Assay: CV<10%

Inter-plate Precision

3 samples with low, middle and high level SARS-CoV-2 Spike S1 Protein Antibody were tested on 3 different plates, 20 replicates in each plate.

Inter-Assay: CV<15%

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean(ng/mL)	1.95	6.25	37.5	1.95	6.25	37.5
Standard deviation	0.04	0.17	1.24	0.12	0.43	1.61
CV(%)	2.1	2.7	3.3	6.4	6.8	4.3

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of SARS-CoV-2 Spike S1 Protein Antibody and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

/	/	Cell Culture Media(n=5)	Serum(n=5)
1:2	Average of Expected（%）	96	100
	Range（%）	90-102	92-108
1:4	Average of Expected（%）	97	100
	Range（%）	88-106	85-115
1:8	Average of Expected（%）	102	103
	Range（%）	93-111	96-110
1:16	Average of Expected（%）	105	106
	Range（%）	95-114	98-114

Trouble Shooting

Problem	Possible Cause	Solution
High Background	Insufficient washing	Sufficiently wash plates as required. Ensure appropriate duration and number of washes. Ensure appropriate volume of wash buffer in each well.
	Incorrect incubation procedure	Check whether the duration and temperature of incubation are set up as required.
	Cross-contamination of samples and reagents	Be careful of the operations that could cause cross-contamination. Use fresh reagents and repeat the tests.
No signal or weak signal	Incorrect use of reagents	Check the concentration and dilution ratio of reagents. Make sure to use reagents in proper order.
	Incorrect use of microplate reader	Warm the reader up before use. Make sure to set up appropriate main wavelength and correction wavelength.
	Insufficient colour reaction time	Optimum duration of colour reaction should be limited to 15-25 minutes.

	Read too late after stopping the colour reaction	Read the plate in 5 minutes after stopping the reaction.
	Matrix effect of samples	Use positive control.
Too much signal	Contamination of TMB substrate	Check if TMB substrate solution turns blue. Use new TMB substrate solution.
	Plate sealers reused	Use a fresh new sealer in each step of experiments.
	Protein concentration in sample is too high	Do pre-test and dilute samples in optimum dilution ratio.
Poor Duplicates	Uneven addition of samples	Check the pipette. Periodically calibrate the pipette.
	Impurities and precipitates in samples	Centrifuge samples before use.
	Inadequate mixing of reagents	Mix all samples and reagents well before loading.

*For research purposes only. Not for therapeutic or diagnostic purposes.