SARS-CoV-2 Nucleocapsid Protein IgG Antibody ELISA Kit

Catalog No.: RK04139

version: 2.0

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

Introduction

The kit applies for detecting the titer of SARS-CoV-2 Nucleocapsid Protein IgG Antibody in serum and plasma. Only used for qualitative tests, not for quantitative tests.

Principle of the Assay

This assay employs the indirect immunoassay technique. A SARS-CoV-2 Nucleocapsid has been pre-coated onto a microplate. Antibodies and samples are pipetted into the wells and any SARS-CoV-2 Nucleocapsid Protein IgG Antibody present is bound by the immobilized protein. After incubation unbound samples are removed during a wash step, and then a secondary antibody is added to the wells and binds to the combination of capture protein and SARS-CoV-2 Nucleocapsid Protein IgG Antibody in sample. Following a wash to remove any unbound combination, TMB substrate is added. This chromogenic substrate formed in proportion to the amount of SARS-CoV-2 Nucleocapsid Protein IgG Antibody present in the sample. The reaction is terminated by acid and the absorbance is measured.

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
Antigen Microwell Plate Coated	8×12	RM17517	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.
Control Antibody (100×)	1x20 μL	RM17518	It is not recommended to use again after redissolving.
Concentrated Secondary Antibody (1000×)	1 ×30 μL	RM17519	Store at 2-8°c for 1 month *

Control/Sample Diluent (R1) (4x)	1 ×20mL	RM00023	Store at 2-8°c for 1 month *
Secondary Antibody Diluent (R2)	1 ×12mL	RM00024	
Wash Buffer (20x)	1 ×30mL	RM00026	
TMB Substrate	1 ×12 mL	RM00027	
Stop Solution	1 ×6 mL	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 xg. 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

1. 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.

2. **Control/Sample Diluent (R1 4x)** -Dilute 1:4 with double distilled or deionized water before use, for example : Add 5 mL of Standard/Sample Diluent Concentrate to 15mL of deionized or distilled water to prepare 20 mL of Control/Sample Diluent .

3. **Control Antibody (100x):**Dilute 1:100 with the 1x Control/Sample Diluent(R1 1x), sit for a minimum of 15 minutes with gentle agitation.

4. **Concentrated Secondary Antibody (1000x)**: Dilute 1:1000 with the Secondary Antibody Diluent (R2) before use, and the diluted solution should be used within 30 min.

5. **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.

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 standards, 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 60 seconds, repeating the process two times for a total of three washes.
3. Add 100 μL 1x Control/Sample Diluent （R1 1x）in blank well.
4. Add 100 μL Control Antibody Working Solution or sample in other wells, cover with the adhesive sealer provided. Incubate for 2 hours at 37℃.
5. Repeat the aspiration/wash as in step 2.
6. Prepare the Concentrated Secondary Antibody (1000x) Working Solution 15 minutes early before use.
7. Add Secondary antibody Working Solution in each well (100μL/well), cover with new adhesive sealer provided.

Incubate for 1 hour at 37°C.

8. During the incubation, turn on the microplate reader to warm up for 30 minutes before measuring.
9. Repeat the aspiration/wash as in step 2.
10. Add TMB Substrate (100 μ L/well). Incubate for 15-20 minutes at 37°C. Protect from light.
11. Add Stop Solution (50 μ L/well), 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 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 37℃, then wash 3 times

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Add 100 μL Secondary antibody Working Solution

Incubate for 1 hours at 37℃, then wash 3 times

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Add 100 μL Substrate Solution

Incubate for 15-20 min at 37℃ 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

Specificity

The test recognizes recombinant and natural SARS-CoV-2 Nucleocapsid Protein IgG Antibody .

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.