SARS-CoV-2 Nucleocapsid Protein IgG Antibody ELISA Kit

Catalog NO.:RK04257

version: 2.0

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

Introduction

The kit is a Indirect enzyme immunoassay for in vitro quantitative measurement of SARS-CoV-2 Nucleocapsid Protein IgG Antibody in human serum, plasma.

Principle of the Assay

This assay employs the quantitative Indirect enzyme immunoassay technique. A SARS-CoV-2 Nucleocapsid Protein has been pre-coated onto a microplate. Control Antibodies and samples are pipetted into the wells and any SARS-CoV-2 Nucleocapsid Protein 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 Nucleocapsid Protein IgG Antibody in sample. Following a wash to remove any unbound combination, a TMB substrate is added. This chromogenic substrate formed in proportion to the amount of Nucleocapsid Protein IgG Antibody present in the sample. The reaction is terminated by acid and the absorbance is measured.

Material Provided & Storage Conditions

Store unopened kit at 2-8 ° C. Do not use past kit expiration date.It is highly recommended to use the remaining reagents within 1 month provided.

Part	Size	Cat. No.	Storage of opened/reconstituted material
Antigen Coated Plate	8×12	RM94513	Return unused wells to the foil pouch containing the desiccant pack and store at ≤ -20 ° C. Reseal along entire edge of zip-seal.
Control Antibody (100×)	1x20ul	RM94514	Aliquot and store at ≤ -20 ° C in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Concentrated Secondary Antibody (100×)	1 ×120ul	RM94515	May be stored for up to 6 month at -20 ° C.*
Control/Sample Diluent (R1) (4x)	1 ×20mL	RM00023	May be stored for up to 6 month at 2-8 ° C.
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		

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 FOR USE IN DIAGNOSTIC PROCEDURES.

1. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
2. Variations in sample collection, processing, and storage may cause sample value differences.
3. Reagents may be harmful. If ingested, rinse it with an excess amount of tap water.
4. Stop solution contains strong acid. Wear eye, hand, and face protection.
5. Please perform simple centrifugation to collect the liquid before use.
6. Do not mix or substitute reagents with those from other lots or other sources.
7. Adequate mixing is particularly important for good result. Use a mini-vortexer at the lowest frequency.
8. Mix the sample and all components in the kits adequately and use clean plastic container to prepare all diluents.
9. Both the sample and standard should be assayed in duplicate, and regents should

be added in sequence in accordance with the requirement of the specification.

10. Reuse of dissolved standard is not recommended.
11. The kit should not be used beyond the expiration date on the kit label.
12. The kit should be away from light when it is stored or incubated.
13. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma, and other biological fluids in accordance with NCCLS regulations.
14. To avoid cross contamination, please use disposable pipette tips.
15. Please prepare all the kit components according to the Specification. If the kit will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
16. The 48T kit is also suitable for the specification.

Sample Collection & Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Samples containing the correlated IgG as in this kit may interfere with this assay.

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 after 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.

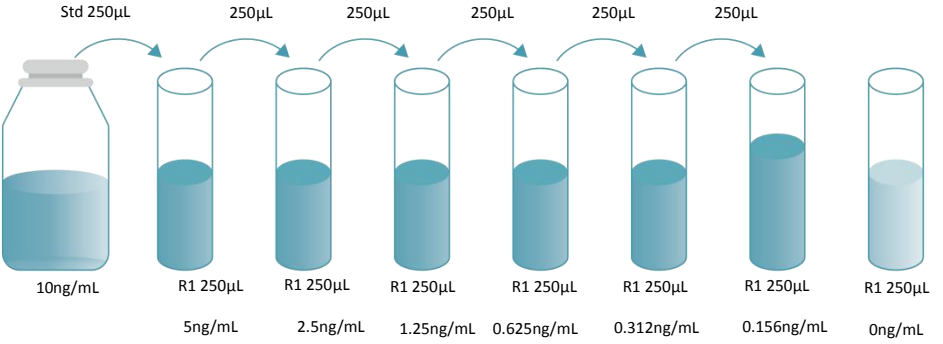
Reagent Preparation

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/Sample Diluent (R1) (4x)：Dilute 1:4 with the PBS before use.

Control Antibody: - Dilute 1:100 with the 1x Control/Sample Diluent(R1). This reconstitution produces a stock solution of 10ng/mL. Mix the Antibody to ensure complete reconstitution.

Use the 10ng/mL Antibody stock to produce a dilution series (below) with 1x Control/Sample Diluent(R1). Mix each tube thoroughly and change pipette tips between each transfer (recommended concentration for standard curve: 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0ng/mL). Use diluted standards within 60 minutes of preparation.



Concentrated Secondary Antibody (100x): Dilute 1:100 with the Secondary Antibody Diluent (R2) before use, and the diluted solution should be used within 30 min.

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

Note During the whole procedure, it is recommended that a face mask and gloves

be used to protect kit reagents from contamination.

Sample - Different types of sample need appropriate dilutions.

Serum (pretreated) and plasma samples require a 10000-fold dilution with 1x Control/Sample Diluent(R1).

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 Control antibodies, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add wash buffer 350 μL/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes.
4. Add 100 μL 1x Control/Sample Diluent (R1) in a blank well.
5. Add 100 μL different concentration of Control Antibodies and samples in other wells, Cover with the adhesive strip provided. Incubate for 2 hours at 37℃. record the plate layout of standards and sample assay.
6. Prepare the Concentrated Secondary Antibody (100x) Working Solution 15 minutes

early before use.

7. Repeat the aspiration/wash as in step 3.
8. Add Secondary antibody Working Solution in each well (100μL/well), cover with new adhesive sealer provided. Incubate for 1 hour at 37° C.
9. During the incubation, turn on the microplate reader to warm up.
10. Repeat the aspiration/wash as in step 3.
11. Add 100 μL TMB Substrate to each well. Incubate for 15-20 minutes at 37℃ .Protect from light.
12. 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 be higher and less accurate.

Assay Procedure Summary

Prepare the standard and reagents

wash 3 times

↓

Add 100ul of antibodies and test samples to each well

Incubate for 2 hours at 37℃, then wash 3 times

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Add 100ul Secondary antibody Working Solution

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

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Add 100ul Substrate Solution

Incubate for 15-20 min at 37℃ under dark condition

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Add 50ul Stop Solution

↓

Detect the optical density within 5 minutes under 450nm.

Correction Wavelength set at 570nm or 630nm

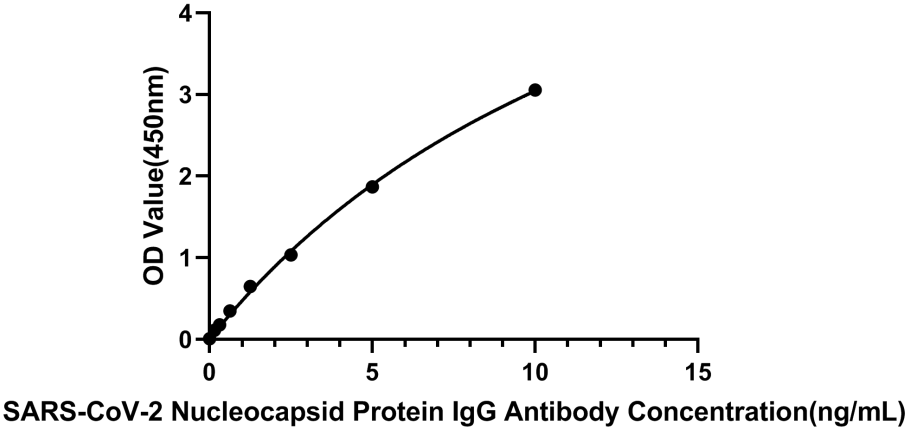
Calculation of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).

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 Nucleocapsid Protein IgG 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 Nucleocapsid Protein IgG Antibody assayed.

Sensitivity

~~The minimum detectable dos~~e (MDD) of SARS-CoV-2 Nucleocapsid Protein IgG Antibody typically less than 0.069ng/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.

Specificity

This assay recognizes SARS-CoV-2 Nucleocapsid Protein IgG Antibody.

Note:

Limited by current skills and knowledge, it is impossible for us to complete the cross- reactivity detection between SARS-CoV-2 Nucleocapsid Protein IgG Antibody and all the analogues, therefore, cross reaction may still exist.

Precision

~~Intra-plate Precision~~

3 samples with low, middle and high level SARS-CoV-2 Nucleocapsid Protein IgG Antibody were tested 16 times on one plate, respectively.

Intra-Assay: CV<10%

Inter-plate Precision

3 samples with low, middle and high level SARS-CoV-2 Nucleocapsid Protein IgG Antibody were tested on 3 different plates, 8 replicates in each plate.

Inter-Assay: CV<15%

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(ng/mL)	0.51	2.01	8.72	0.54	2.08	8.65
Standard deviation	0.02	0.11	0.50	0.03	0.14	0.67
CV(%)	4.2	5.4	5.7	6.1	6.8	7.8

Recovery

Matrices listed below were spiked with certain level of SARS-CoV-2 Nucleocapsid Protein IgG Antibody and the recovery rates were calculated by comparing the measured value to the expected amount of SARS-CoV-2 Nucleocapsid Protein IgG Antibody in samples.

Problem	Possible Cause	Solution

Sample	Average Reovery (%)	Range (%)
Serum(n=5)	106	94-114

Linearity

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

/	/	Serum(n=5)
1:2	Average of Expected (%)	85
	Range (%)	81-95
1:4	Average of Expected (%)	91
	Range (%)	89-98
1:8	Average of Expected (%)	98
	Range (%)	95-107
1:16	Average of Expected (%)	101
	Range (%)	98-104

Trouble Shooting

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.