

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 anti-SARS-CoV-2 Nucleocapsid Protein IgG Antibody in serum and plasma.

Principle of the Assay

This assay employs the Indirect immunoassay technique. A Nucleocapsid Protein specific for Nucleocapsid Protein Antibody has been pre-coated onto a microplate. Antibodies and samples are pipetted into the wells and any Nucleocapsid Protein Antibody present is bound by the immobilized protein. Following 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-Nucleocapsid Protein Antibody in sample. Following a wash to remove any unbound combination, a substrate is added. A colored product TMB is formed in proportion to the amount of Nucleocapsid Protein Antibody present in the sample. The reaction is terminated by addition of acid and 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 this is prior to the expiration date of the kit.

Part	Size (96T)	Cat NO.	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Antigen Coated Plate	8x12	RM17517	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(100x)	1x20ul	RM17518	May be stored for up to 6 month at -20°C.*
Concentrated Secondary Antibody (1000x)	1 x30ul	RM17519	May be stored for up to 6 month at -20 °C.*
Control/Sample Diluent (R1)(4x)	1 x20mL	RM00023	May be stored for up to 6 month at 2-8 °C.*
Secondary Antibody Diluent (R2)	1 x12mL	RM00024	
Wash Buffer(20x)	1 x 30mL	RM00026	
TMB Substrate	1 x12 mL	RM00027	
Stop Solution	1 x6 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

1. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
2. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
3. Variations in sample collection, processing, and storage may cause sample value differences.
4. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
5. Stop Solution contains strong acid. Wear eye, hand, and face protection.
6. Apart from the standard of kits, other components should not be refrigerated.
7. Please perform simple centrifugation to collect the liquid before use.
8. Do not mix or substitute reagents with those from other lots or other sources.

9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
10. Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all of the diluent.
11. Both the sample and standard should be assayed in duplicate, and the sequence of the reagents should be added consistently.
12. Reuse of dissolved standard is not recommended.
13. The kit should not be used beyond the expiration date on the kit label.
14. The kit should be away from light when it is stored or incubated.
15. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
16. To avoid cross contamination, please use disposable pipette tips.
17. 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 2 months.
18. This assay is designed to eliminate interference by other factors present in biological samples.
19. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.
20. 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 $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma : Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. 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 1:100 with the 1xControl/Sample Diluent(R1) , sit for a minimum of 15 minutes with gentle agitation.

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.

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. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Add wash buffer 350 μ L/well, aspirate each well after holding 60 seconds, repeating the process two times for a total of three washes.
3. Add 100 μ L Control/Sample Diluent (R1) in blank well.
4. Add 100 μ L Control Antibody Working Solution and sample in other wells, cover with the adhesive strip provided. Incubate for 2 hours at 37°C.
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 wells (100 μ L/well), cover with new adhesive strip provided. Incubate for 1 hour at 37°C.
8. Warm-up the Microplate reader.
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 be higher and less accurate.

Assay Procedure Summary

Prepare the reagents



Add 100ul of Control Antibody and test samples to each well

Incubate for 2 hours at 37°C, then wash 3 times



Add 100ul Secondary antibody Working Solution

Incubate for 1 hour at 37°C, then wash 3times



Add 100ul Substrate Solution

Incubate for 15-20 min at 37°C under dark condition



Add 50ul Stop Solution



Detect the optical density within 5 minutes under 450nm.

Correction Wavelength set at 570nm or 630nm

Specificity

This assay recognizes both recombinant and natural Nucleocapsid Protein IgG Antibody.

Precision

Intra-plate Precision

3 samples with low, middle and high level Nucleocapsid Protein were tested 20 times on one plate, respectively.

Intra-Assay: CV<10%

Inter-plate Precision

3 samples with low, middle and high level Nucleocapsid Protein were tested on 3 different plates, 8 replicates in each plate.

Inter-Assay: CV<12%

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean(ng/mL)	35	105	260	17	114	416
Standard deviation	1.12	4.1	10.7	1.2	7.9	26.6
CV(%)	3.2	3.9	4.1	7.1	6.9	6.4

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.