SARS-CoV-2 Inhibitor Screening Kit（RBD）

Catalog NO.: RK04149

version: 2.0

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

Introduction

This ELISA kit used for quantitative determination of RBD Protein Neutralizing Antibody in human serum and plasma. For research use only, and it's highly recommended to read thoroughly of this manual before using the product.

Principle of the Assay

This assay employs the competitive inhibition enzyme immunoassay technique. A RBD protein specific to RBD Neutralizing Antibody has been pre-coated onto a microplate. A competitive inhibition reaction is launched between Biotin labeled ACE2 protein and RBD Neutralizing Antibody (Standards or samples) with the pre-coated RBD protein specific to RBD Neutralizing Antibody. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps, a substrate is added. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of RBD Neutralizing Antibody in the sample.

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	Cat. No.	Storage of opened/reconstituted material
Antigen Coated Plate	8×12	RM41000	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(50×)	1 ×20μL	RM41001	May be stored for up to 6 month at -20 °C.*
Concentrated Biotin-Conjugate ACE2(100×)	1 ×120μL	RM41002	May be stored for up to 6 month at -20 °C.*
Streptavidin-HRP Concentrated (100x)	1 ×120μL	RM41003	May be stored for up to 6 month at 2-8 °C.*
Control/Sample Diluent (R1)	1 ×20mL	RM00023	May be stored for up to 6 month at 2-8 °C.*
Biotin- Conjugate ACE2 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		

*Provided this is within the expiration date of the kit.

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 regents 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.

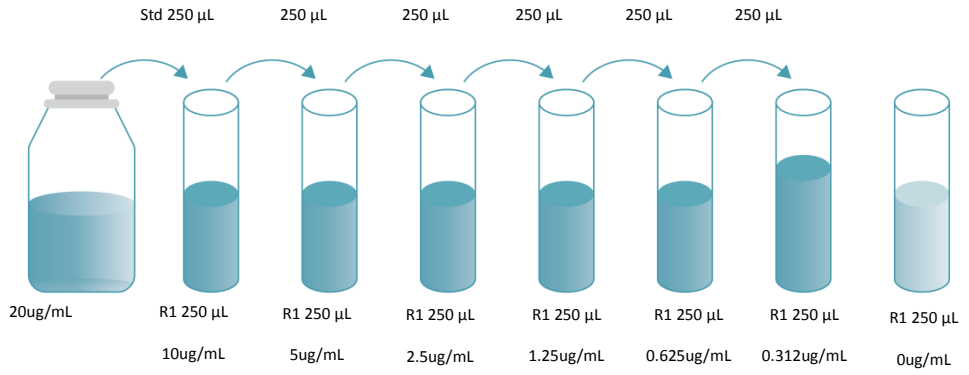
Serum : Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 ℃. Avoid repeated freeze-thaw cycles.
Plasma : Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of coolection. 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 1:50 with the Antibody/Sample Diluent(R1) , sit for a minimum of 15 minutes with gentle agitation prior to making dilutions (20ug/mL), Prepare EP tubes containing Antibody/Sample Diluent(R1), and produce a dilution series according to the picture shown below (recommended concentration for standard curve: 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0ug/mL). Redissolved antibody solution (20ug/mL), aliquot and store at -20℃— -70℃.



Working Biotin-Conjugate ACE2 : Dilute 1:100 of Concentrated Biotin-Conjugate ACE2 (100x) with Biotin- Conjugate ACE2 Diluent (R2) before use, for example: Add 20 µL of Concentrated Biotin-Conjugate ACE2 (100x) to 1980 µL Biotin- Conjugate ACE2 Diluent (R2) to prepare 2000 µL Working Biotin-Conjugate ACE2 Buffer.

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

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all 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. Prepare the Biotin-Conjugate ACE2 Concentrated (100X) Working Solution 15 minutes early before use.
3. Add 50 µL Control/Sample Diluent (R1) in blank well.
4. Add 50 µL different concentration of Control Antibody and samples in other wells, Add then add Biotin-Conjugate ACE2 Working Solution in each wells (50 µL/well), cover with new adhesive strip provided. Incubate for 1.5 hours at 37° C.
5. Remove the liquid of each well, Add wash buffer 350 µL/well, aspirate each well after holding 60-120 seconds, repeating the process two times for a total of three washes.
6. Add Streptavidin-HRP Working Solution in each wells (100 µL/well), cover with new adhesive strip provided. Incubate for 30 minutes at 37°C.
7. Warm-up the Microplate reader.
8. Repeat the aspiration/wash as in step 5.
9. Add TMB Substrate (100µL/well). Incubate for 15-20 minutes at 37°C .Protect from light.

10. Add Stop Solution (50μL/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 reagent

↓

Add 50ul of Control Antibody and test samples to other well

↓

Add 50ul Biotin-Conjugate ACE2 Working Solution↓

Incubate for 1.5 hour at 37℃, then wash 3 times

↓

Add 100ul Streptavidin-HRP Working Solution

Incubate for 30 min at 37℃, then wash 3 times

↓

Add 100ul Substrate Solution

Incubate for 10-15 min at 37℃ under dark condition

↓

Add 50ul Stop Solution

↓

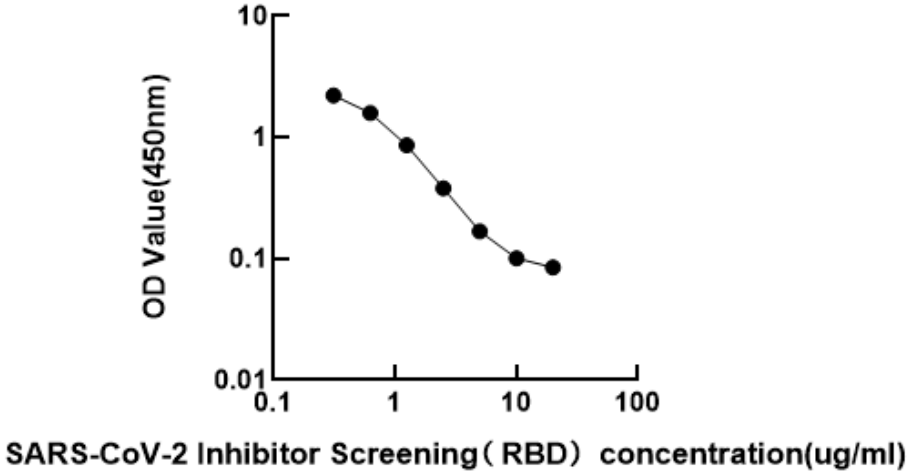
Detect the optical density within 5 minutes under 450nm.

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 RBD Protein Neutralizing 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 RBD Protein Neutralizing Antibody assayed.

Sensitivity

The minimum detectable dose (MDD) of RBD Protein Neutralizing Antibody typically less than 0.112ug/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 RBD Protein Neutralizing Antibody.No significant cross-reactivity was observed.

Note:

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

Precision

Intra-plate Precision

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

Intra-Assay: CV<10%

Inter-plate Precision

3 samples with low, middle and high level RBD Protein Neutralizing 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	20	20	20	20	20	20

Mean(ug/mL)	0.8	1.1	1.5	0.5	0.9	1.6
Standard deviation	0.04	0.06	0.09	0.03	0.06	0.13
CV(%)	5.1	5.6	6.3	6.8	7.2	8.3

Problem	Possible Cause	Solution

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.