SARS-CoV-2 Inhibitor Screening Kit (RBD)

SKU: CBK4149

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

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Introduction

The kit is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of RBD Neutralizing Antibody in serum, plasma.

Principal 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 ACE-2 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.

Materials Provided

Part	Size	Storage of opened/Recon
Protein Coated Plate	8 x 12	Return unused wells to the foil pouch containing the desiccant pack and store at ≤ -20 °C. Reseal along entire edge of zip-seal.
RBD Neutralizing Antibody(50x)	1x 20ul	May be stored for up to 1 month at 2-8°C.*
Concentrated Biotin-Conjugate ACE-2(100×)	1 ×120ul	May be stored for up to 1 month at 2-8°C.*
Streptavidin-HRP Concentrated (100x)	1 ×120ul	May be stored for up to 6 month at 2-8 °C.*
Antibody/Sample Diluent (R1)	1 x 20mL	
Biotin- Conjugate ACE-2 Diluent (R2)	1 x 12mL	
Streptavidin-HRP Diluent(R3)	1 x 12mL	
Wash Buffer (20x)	1 × 30mL	
TMB Substrate	1 ×12 mL	
Stop Solution	1 ×6 mL	
Plate Sealers	4 strips	
Specification	1	

Sample Collection And Storage

1. Serum:

Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1,000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

2. Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles**.**

3. Avoid hemolytic and hyperlipidemia sample for Serum and Plasma.

4. Dilution:

Dilute samples at the appropriate multiple (recommend to do pre-test to determine the dilution factor).

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.

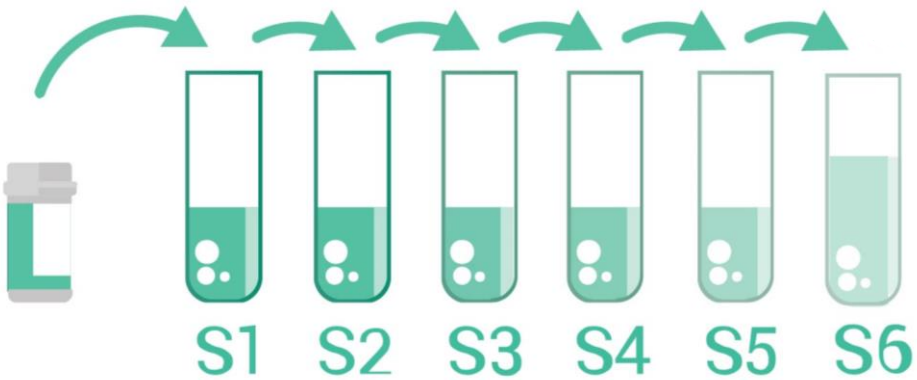
18. The 48T kit is also suitable for the specification.

Experimental Materials

1. Microplate reader(measuring absorbance at 450 nm, with the correction wavelength set at 570 nm or 630 nm).
2. Pipettes and pipette tips：0.5-10, 2-20, 20-200, 200-1000 µL.
3. Microplate washer, Squirt bottle.
4. Micro-oscillator.
5. Deionized or double distilled water, graduated cylinder.
6. Polypropylene Test tubes for dilution.
7. Incubator.

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. **RBD Neutralizing 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°C— -70°C.



3. **Concentrated Biotin-Conjugate ACE-2 (100x):** Dilute to the working concentration with Biotin-Conjugate ACE-2 Diluent (R2)

Dilution Method

Strip	Concentrated Secondary Antibody(500x)	Secondary Antibody Diluent (R2)
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

4. **Streptavidin-HRP Concentrated (100x):** Dilute 1:100 with the Streptavidin-HRP Diluent(R3) before use, and the diluted solution should be used within 30 min.

Dilution Method

Strip	Concentrated Secondary Antibody(500x)	Secondary Antibody Diluent (R2)
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

5. **Wash buffer:** Dilute 1:20 with double distilled or deionized water before use.

Wash Method

Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer(300ul) using a squirt bottle, manifold dispenser, or auto-washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining **Wash Buffer** by aspirating or decanting. Invert the plate and blot it against clean paper towels.

Assay Procedure

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 ACE-2 Concentrated (100X) Working Solution 15 minutes early before use.

3. Add 50 µL Antibody/sample Diluent (R1) in blank well.

4. Add 50 µL different concentration of Antibodies and samples in other wells, Add then add Biotin-Conjugate ACE-2 Working Solution in each wells (50µL/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 four times for a total of five 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.

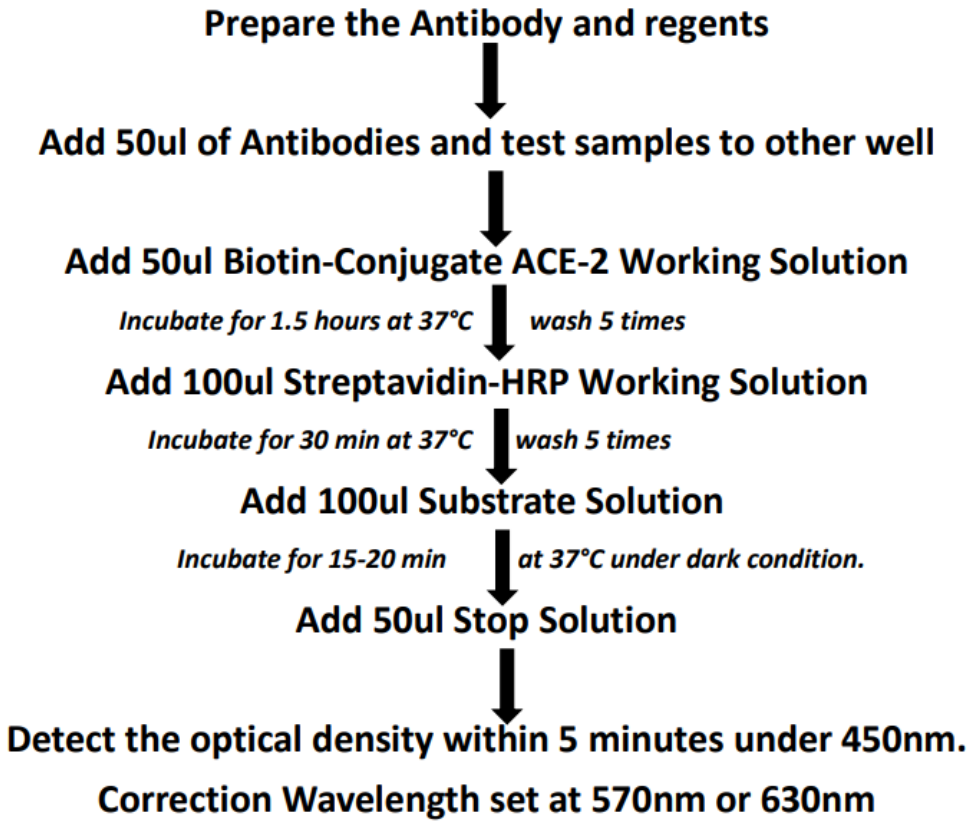
7. Warm-up the Microplate reader.

8. Repeat the aspiration/wash as in step 5.

9. Add TMB Substrate (100µL/well). Incubate for 15-20 minutes at 11 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



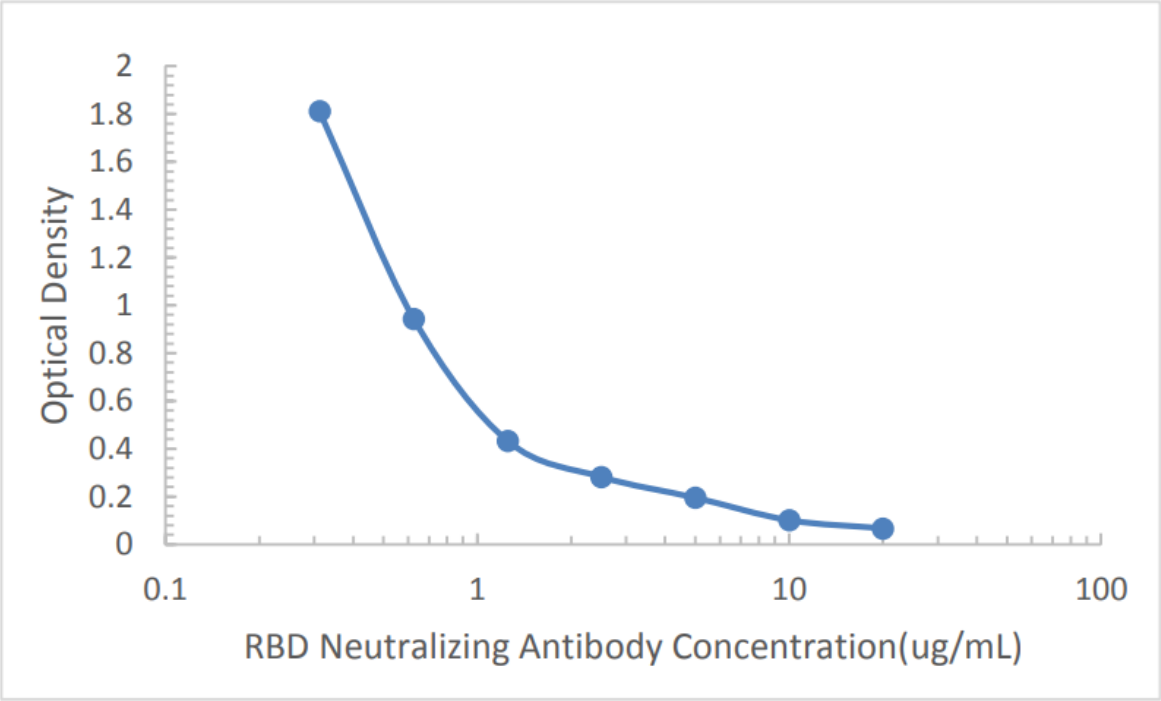
Calculation of Results

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 log/log 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 Spike RBD 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 Spike RBD Protein IgG Antibody assayed.

Sensitivity

The minimum detectable dose (MDD) of RBD Neutralizing Antibody is 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.

Precision

Intra-plate Precision

3 samples with low, middle and high level RBD Neutralizing Antibody were tested 20 times on one plate, respectively. Intra-Assay: CV

Inter-plate Precision

3 samples with low, middle and high level RBD Neutralizing Antibody were tested on 3 different plates, 8 replicates in each plate. Inter-Assay: CV<12%

Notes:

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