AssayGenie

Technical Manual

SARS-COV-2 Spike RBD Protein Antibody ELISA Kit

- Catalogue Code: CBK4144
- Sandwich ELISA Kit
- Research Use Only

Contents

Principle of the Assay

This assay employs a quantitative sandwich enzyme immunoassay technique. A Recombinant Recombinant SARS-COV-2 Spike RBD Protein has been pre-coated onto a microplate. To perform the assay, Control Antibody and samples are pipetted into the wells and samples are bound by the immobilized Protein. Following incubation, unbound antibodies are removed during a wash step, and then a detection protein is added to the wells and binds to the combination of Spike RBD Protein-Control antibody in the sample. Following a wash to remove any unbound combination, an enzyme conjugate is added to the wells. Following incubation and wash steps, a substrate is added. A coloured TMB product is formed in proportion to the amount of Spike RBD Protein Antibody present in the sample. The reaction is terminated by addition of acid and absorbance is measured. A standard curve is prepared from seven Control Antibody standard dilutions and Spike RBD Protein Antibody concentration determined.

Materials Provided and Storage Conditions

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

Part	Size	Storage
Recombinant SARS-COV-2 Spike RBD 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.
Control Antibody (1000×)	1x40ul	Aliquot and store at -20 °C. Avoid repeated freeze-thaw cycles.
Concentrated Biotin Conjugate Antigen (250x)	1 × 40ul	May be stored for up to 6 months at -20°C.
Streptavidin-HRP Concentrated (100x)	1 ×120ul	May be stored for up to 6 months at 2-8 °C.
Control/Sample Diluent (R1)	1 ×20mL	May be stored for up to 6 months at 2-8 °C.
Biotin-Conjugate Protein 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	
Manual	1	

Other materials required but not supplied:

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630nm or 570nm.
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 and if ingested, rinse 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 particularly important for good results. Use a mini-vortexer at the lowest frequency.
10. Mix the sample and all components in the kits adequately and use clean plastic containers to prepare all of the diluent.
11. Both the sample and standard should be assayed in duplicate, and reagents should be added in sequence in accordance with the requirement of the specification.
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, use disposable pipette tips.
17. Please prepare all the kit components according to the specifications. 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 and Storage:

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

Samples containing the corrected 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 before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C. Avoid freeze/thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection and detect. Assay immediately or aliquot and store samples at -20°C. Avoid freeze / thaw cycles. (Note: Citrate plasma has not been validated for use in this assay)

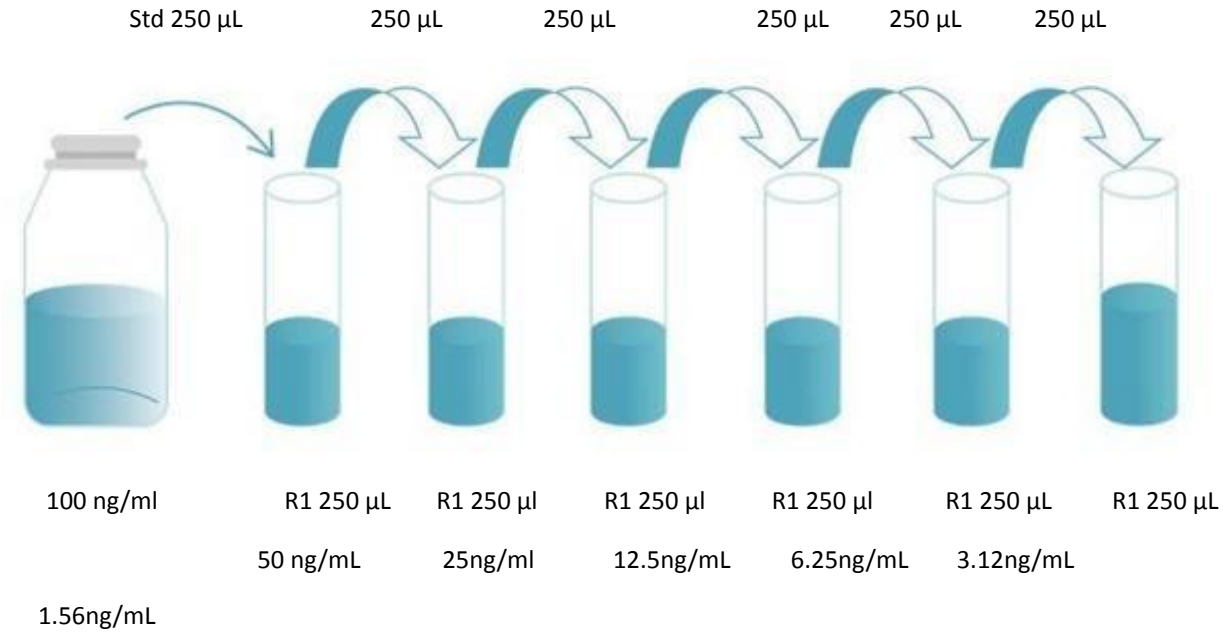
Other biological fluids: Centrifuge samples for 20 minutes at 1,000xg. Collect supernates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid freeze / thaw cycles.

Notes: It is suggested that all samples in this 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 1:1000 with the Control/Sample Diluent (R1) and incubate for a minimum of 15 minutes with gentle agitation prior to making dilutions (100ng/mL). Prepare EP tubes containing Control/Sample Diluent (R1) and produce a dilution series according to the picture shown below (recommended concentration for standard curve: 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0ng/mL). Redissolved standard solution (100ng/mL), aliquot and store at -20°C to -70°C.



Concentrated Biotin Conjugate Antigen (250x): Dilute 1:250 with the Biotin-Conjugate Antibody (250x) with Biotin-Conjugate Antigen Diluent (R2) before use, and the diluted solution should be used within 30 min.

Working Streptavidin-HRP: Dilute 1:100 of Concentrated Streptavidin-HRP (100x) with Streptavidin-HRP diluent (R3) before use, for example: add 20µl of Concentrated Streptavidin-HRP (100x) to 1980µl of Streptavidin-HRP diluent (R3) to prepare 2000µl 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 eater before use, for example: add 20mL of Wash Buffer Concentrated to 380mL of deionized or distilled water to prepare 400mL of Wash Buffer.

Assay Procedure

Bring all reagents to room temperature before use. If is recommended that all standards, controls and samples be assayed in duplicate. Prepare all reagents, working controls and samples as directed in the previous sections.

1. Remove excess microplate strips from the plate frame and return them to the foil pouch containing the desiccant pack and reseal.

2. Add wash buffer 350 µL/well, aspirate each well after holding for 40 seconds. Repeat the process two times for a total of three washes.

3. Add 100 µL Control/Sample Diluent(R1) to the blank well.

4. Add 100 µL of controls and samples to the appropriate wells and cover with the adhesive strip provided. Shake with Micro-oscillator (250 r/min). Incubate for 2 hours at room temperature. Note: Record the plate layout of standards and sample assay.

5. Prepare the **Concentrated Biotin Conjugate Antigen (250X)** Working Solution 15 minutes early before use.

6. Repeat the aspiration/wash as in step 2.

7. Add 100ul of Working Biotin Conjugate Antigen in each well cover with new adhesive strip provided. Shake with Micro-oscillator (250 r/min). Incubate, while shaking for 1 hour at room temperature.

8. Prepare the **Streptavidin-HRP Concentrated** (100X) Working Solution 15 minutes before use.

9. Repeat the aspiration/wash as in step 2.

10. Add Streptavidin-HRP Working Solution to each well (100 µL/well) and cover with a new adhesive strip. Shake with Micro-oscillator (250 r/min). Incubate, while shaking for 30 minutes at room temperature.

11. Warm up the Microplate reader.

12. Repeat the aspiration/wash as in step 2.

13. Add TMB Substrate (100µL/well). Incubate for 15-20 minutes at room temperature and protect from light.

14. 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

Wash standards and reagents
Wash 3 times

Add 100ul of Control and test samples to each well

Incubate for 2 hours at RT, then wash 3 times

Add 100ul Biotin-Conjugate Protein Working Solution

Incubate for 1 jour at RT, then wash 3 times

Add 100ul Working Streptavidin-HRP

Incubate for 30 minutes at RT, then wash 3 times

Add 100ul Substarte Solution

Incubate for 15-20 min at RT, under dark condition

Add 50ul Stop Solution

Detect the optical within 5 minutes under 450nm.

Correction Wavelength sat 570nm or 630nm.

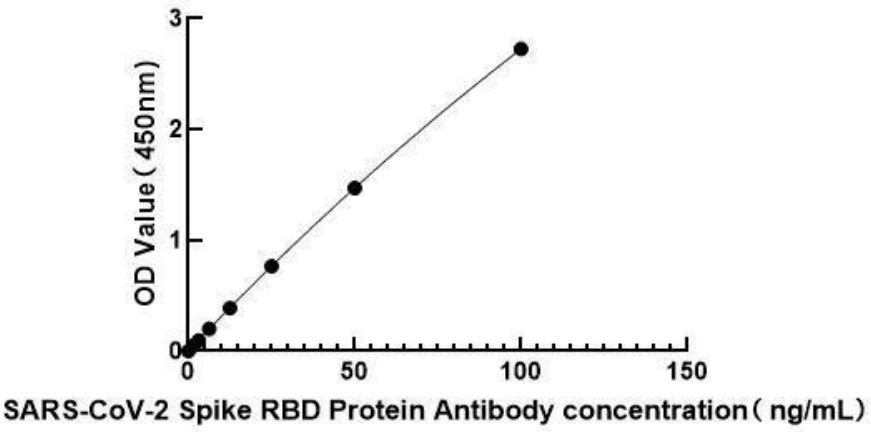
Calculation of Results

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 Spike RBD Protein 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 assayed.

Sensitivity

The minimum detectable dose (MDD) of Spike RBD Protein Antibody is typically less than 163.1pg/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

Three samples of low, middle and high-level Spike RBD Protein were tested 20 times on one plate.
Intra-Assay: CV<10%
Inter-plate Precision

Three samples of low, middle and high level Spike RBD Protein were tested on 3 different plates, 20 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 (pg/mL)	15	57	86	24	36	79
Standard Deviation	0.57	2.74	4.73	1.51	2.52	6.40
CV (%)	3.8	4.8	5.5	6.3	7.0.	8.1

Linearity

The linearity if the kit was assayed by testing samples spiked with appropriate concentrations of Spike RBD protein and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to be expected.

Dilution	/	Cell Culture Media (n=5)	Serum (n=5)
1:2	Average of Expected (%)	92	96
	Range (%)	82-102	86-106
1:4	Average of Expected (%)	99	99
	Range (%)	91-107	88-109
1:8	Average of Expected (%)	96	106
	Range (%)	83-109	95-117
1:16	Average of Expected (%)	103	97
	Range (%)	90-115	84-110

Trouble Shooting

Problem	Possible Cause	Solution
High Background	Insufficient washing	Sufficiently wash plate 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 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	Warm the reader up before use. Make sure to set up appropriate maim wavelength and correct wavelength
	Insufficient colour reaction tome	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 experiment.
	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 precipitate 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.

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