FT-B8DZZ0

SARS-COV-2 Spike S1 Protein IgG Antibody ELISA Kit

Catalog Number: RK04138

This ELISA kit used for quantitative determination of 2019-nCoV Spike S1 Protein IgG Antibody concentrations in serum and plasma. For research use only, and it's highly recommended to read throughly of this manual before using the product.

Introduction

The kit applies for detecting the level of anti-SARS-CoV-2 (2019-nCoV) Spike S1 Protein IgG antibodies in serum and plasma.

Principle Of The Assay

This assay employs the Indirect immunoassay technique. A Spike S1 Protein specific for Spike S1 Protein Antibody has been pre-coated onto a microplate. Antibodies and samples are pipetted into the wells and any Spike S1 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-Spike S1 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 Spike S1 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 Spike S1 Protein Antibody standard dilutions and Spike S1 Protein Antibody sample concentration determined.

Materials Provided

Unopened Kit: Store at 2 - 8°C and the kit is stable for 3 months upon receipt.

Part	Size (96T)	Cat NO.	STORAGE OF OPENED/RECONSTITUTED MATERIAL
Antigen Coated Plate	8×12	RM17513	Return unused wells to the foil pouch containing the desiccant pack and store at 2-8 °C.Reseal along entire edge of zip-seal.
Control Antibody(100×)	1x20μL	RM17514	May be stored for up to 1 month at 2-8 °C.
Concentrated Secondary Antibody (1000×)	1 ×30uL	RM17515	May be stored for up to 1 month at 2-8 °C.
Standard/Sample Diluent (R1)	1 ×20mL	RM00023	May be stored for up to 6 month at 2-8 °C.*
Biotin-Conjugate 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		

Sample Collection And Storage

1. Serum:

Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000x g, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.

2. Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid 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.

Experiment Materials

1. Microplate reader(measuring absorbance at 450nm, with the correction wavelength set at 570nm or 630nm).
2. Pipettes and pipette tips: 0.5-10, 2-20, 20-200, 200-1000μL.
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. **Control Antibody:** Dilute 1:100 with the 1xControl/Sample Diluent(R1) , sit for a minimum of 15 minutes with gentle agitation.
3. **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.

Dilution Method

Strip	Concentrated Secondary Antibody (1000x)	Secondary Antibody Diluent (R2)
2	5μL	4995μL
4	10μL	9990μL
6	15μL	14985μL
8	20μL	19980μL
10	25μL	24975μL
12	30μL	29970μL

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

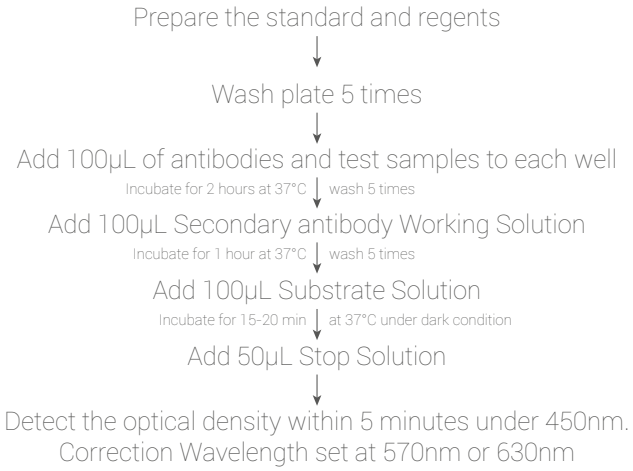
Wash Method

Aspirate each well and wash, repeating the process two times for a total of three washes.Wash by filling each well with **Wash Buffer** (300μL) using a squirt bottle,manifold dispenser,or autowasher. 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. Add wash buffer 300 μL/well, aspirate each well after holding 60 seconds, repeating the process four times for a total of five washes.
3. Add 100 uL 1xControl/Sample Diluent (R1) in blank well.
4. Add 100 μL 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 (100μL/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 (100μL/well). Incubate for 15-20 minutes at 37°C .Protect from light.
11. 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 S1 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.

Specificity

This assay recognizes both recombinant and natural Spike S1 Protein IgG Antibody.

Precision

Inter-plate Precision

Three samples of known concentration were tested 20 times on one plate to evaluate the Intra-plate precision.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	310	1050	2015
Standard Deviation (SD)	11.5	43.1	94.7
Variable Coefficient CV (%)	3.7	4.1	4.7

Inter-plate Precision

Three samples of known concentration were tested 20 times separate assays to evaluate the Inter-plate precision.Assay were using two lots of components.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	321	1071	2013
Standard Deviation (SD)	22.5	71.7	130.8
Variable Coefficient CV (%)	7.0	6.7	6.5