

FT-B8DZY0



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

Catalog Number: RK04137

This ELISA kit used for quantitative determination of 2019-nCoV Spike RBD 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 RBD Protein IgG antibodies in serum and plasma..

Principle Of The Assay

This assay employs the Indirect immunoassay technique. A Spike RBD Protein specific for Spike RBD Protein Antibody has been pre-coated onto a microplate. Antibodies and samples are pipetted into the wells and any Spike RBD 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 RBD 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 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 Spike RBD Protein Antibody standard dilutions and Spike RBD 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
Antibody Coated Plate	8×12	RM17509	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×) 1x20ul RM17510	2	RM17510	May be stored for up to 1 month at 2-8 °C..
Concentrated Secondary Antibody (1000×)	1 ×30uL	RM17511	May be stored for up to 1 month at 2-8 °C.
Standard/Sample Diluent (R1)	1 ×20mL	RM00023	
Biotin-Conjugate Antibody Diluent (R2)	1 ×12mL	RM00024	
Wash Buffer(20x)	1 × 30mL	RM00026	May be stored for up to 6 month at 2-8 °C.*
TMB Substrate	1 ×12 mL	RM00027	
Stop Solution 1 ×6 mL	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. Serum samples require about a 100 fold dilution.

3. 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. Plasma samples require about a 100 fold dilution.

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

5. 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.

- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
- Stop Solution contains strong acid. Wear eye, hand, and face protection.
- Apart from the standard of kits, other components should not be refrigerated.
- Please perform simple centrifugation to collect the liquid before use.
- Do not mix or substitute reagents with those from other lots or other sources.
- Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
- Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all of the diluent.
- Both the sample and standard should be assayed in duplicate, and the sequence of the reagents should be added consistently.
- Reuse of dissolved standard is not recommended.
- The kit should not be used beyond the expiration date on the kit label.
- The kit should be away from light when it is stored or incubated.
- To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
- To avoid cross contamination, please use disposable pipette tips.
- Please prepare all the kit components according to the Specification. If the 8 kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
- The 48T kit is also suitable for the specification.

Experiment Materials

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

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 1x Control/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.

Dilution Method

Strip	Concentrated Biotin-Conjugate antibody (100x)	Biotin-Conjugate Antibody Diluent (R2)
2	5 μ L	4995 μ L
4	10 μ L	9990 μ L
6	15 μ L	14985 μ L
8	20 μ L	19980 μ L
10	25 μ L	24975 μ L
12	30 μ L	29970 μ L

- 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** (300 μ L) 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

- Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
- Add wash buffer 300 μ L/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes.
- Add 100 μ L Standard/sample Diluent (R1) in blank well.
- Add 100 μ L different concentration of standard and sample in other wells, cover with the adhesive strip provided. Incubate for 2 hours at 37°C.
- Repeat the aspiration/wash as in step 2.
- Prepare the Concentrated Secondary Antibody (100X) Working Solution 15 minutes early before use.
- Add Biotin-Conjugate antibody Working Solution in each wells (100 μ L/well), cover with new adhesive strip provided. Incubate for 1 hour at 37°C.
- Prepare the Streptavidin-HRP Concentrated (100X) Working Solution 15 minutes early before use.

9. Repeat the aspiration/wash as in step 2.
10. Add Streptavidin-HRP Working Solution in each wells (100µL/well), cover with new adhesive strip provided. Incubate for 30 minutes at 37°C.
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 37°C .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 450nm. If wavelength correction is available, set to 570nm or 630nm. If wavelength correction is not available, subtract readings at 570nm or 630nm from the readings at 450nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450nm 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 Nucleocapsid Protein 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 RBD Protein IgG Antibody.

Precision

Intra-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)	312	1051	2110
Standard Deviation (SD)	12.2	47.3	90.7
Variable Coefficient CV (%)	3.9	4.5	4.3

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)	305	1020	2071
Standard Deviation (SD)	21.6	66.3	122.2
Variable Coefficient CV (%)	7.1	6.5	5.9