



2019-nCoV S1 Human IgA ELISA Kit

Instruction Manual

Catalog No. C19S1-A877

For the detection of Human IgA antibody to
2019-nCoV Spike Protein S1 in human serum or plasma

This assay kit is for research use only – NOT intended for use in diagnostic or therapeutic procedures.

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INTRODUCTION

Background

The severe acute respiratory syndrome related novel coronavirus SARS-CoV-2 has caused the pandemic of the respiratory diseases (COVID-19) around the world in 2020. Researchers worldwide are racing to develop potential vaccines and drugs to fight the new coronavirus, 2019-nCoV acute respiratory disease.

When it comes to the detection of the new coronavirus, people are most familiar with the nucleic acid test that is currently the "gold standard" for diagnosis. Nucleic acid testing, which uses polymerase chain reaction to detect specific nucleic acid sequences in the viral genome to determine whether the subject is infected with the virus at the moment. The antibody test is a "past tense" test. The human body will produce IgM or IgG antibodies after being infected with the virus. Testing these specific antibodies in the serum can determine whether the subject has been infected with the virus. Recessive cases that have been missed by nucleic acid detection will interfere with the assessment of the epidemic and the prevention and control measures. Antibody testing can help determine the true 'penetration' of the new coronavirus in the population and is very important for understanding the virus's true ability to infect. Many countries have stepped up their efforts to detect antibodies to 2019-nCoV. Another goal is to identify people who are already immune to the virus and prepare for future easing lockdown measures.

About this assay

The 2019-nCoV S1 Human IgA ELISA Kit is an enzyme-linked immunosorbent assay for the detection of Human anti-2019-nCoV Spike protein S1 IgA in serum or other biological samples. Based on indirect ELISA principles, the 2019-nCoV S1 Human IgA ELISA employs recombinant nCoV Spike protein RBD coated on a 96-well plate. Controls and samples (with necessary dilution) are pipetted into the wells; the target IgA in the controls and samples binds to the immobilized Spike protein. The wells are washed and the HRP-labeled Detection antibody is then added. After washing away the unbound HRP-antibodies, a colorimetric substrate solution (TMB) is pipetted to the wells. The absorbance of the color at 450 nm is measured. The intensity of color development in the wells is proportional to the amount of target IgA bound.

GENERAL INFORMATION

Materials supplied

Part #	Part	Quantity / Size
H38A-63DG	Anti-Human IgA Fc Detection Antibody, 1X	1 vial x 10 mL
CP03-C19SD	2019-nCoV Spike Protein RBD Coated Plate	1 plate
CS02-506	One Step TMB Substrate	1 vial x 10 mL
E02-09	nCoV Ig Sample Dilution Buffer	2 vials x 10 mL
SS01-09	Stop Solution	1 vial x 6 mL
C19S1A-NEG	IgA Negative Control	1 vial x 1 mL
C19S1A-POS	IgA Positive Control	1 vial x 1 mL
WB20-09	Wash Buffer, 20X	2 vials x 15 mL

Additional materials required

1. Micro plate cover
2. Milli-Q water or other source of pure water
3. Pipettes, or multichannel pipettes
4. Incubator for 37°C incubation
5. Microplate reader capable of reading absorbance at 450 nm

Storage/Stability

The entire kit may be stored at 4°C for up to 6 months. The coated plate is provided as a multistrip assay plate in the kit, allowing the user to select the appropriate number of strips for each experiment. The remaining unused strips should be stored sealed and used within 1 month after opening.

ASSAY WORKFLOW

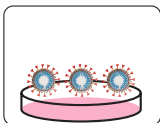
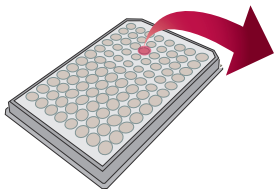
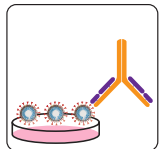
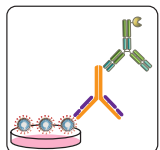


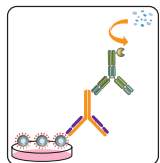
Plate pre-coated with
2019-nCoV Spike protein RBD



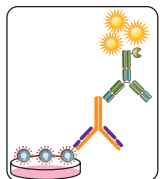
Add sample



Wash plate & add detection antibody



Wash plate & add TMB substrate



Add stop solution & read plate

LEGEND



Spike Protein (RBD)



Sample Antibody



Detection Antibody



TMB Substrate



Oxidized TMB

PROTOCOLS

Reagent preparation

The materials supplied in this kit are sufficient for 100 ELISA assays using the provided protocol. Users may adjust the amounts of reagents prepared according to number of assays needed and store unused reagents at appropriate conditions as indicated.

1. Wash Buffer, 1X

Add 570 mL of Milli-Q water to 30 mL of the 20X Wash Buffer. The diluted wash buffer can be stored at 4 °C for up to a week. For future use, store at -20°C

2. Sample dilution

The serum or plasma samples can be diluted with the sample dilution buffer provided in the kit. The suggested starting dilution is 1:100 for serum or plasma samples. The kit may be able to detect anti-nCoV Spike protein IgA in other biological samples, but this has not been evaluated. It is recommended that several dilutions of samples be performed to determine the optimal dilution.

Assay procedure

Bring all the kit reagents to room temperature before use. Unused microwell strips should be returned to the original re-sealable bag containing the desiccant pack, stored at 4°C, and used within one month of opening.

1. Place the required number of pre-coated strips onto an ELISA plate frame.
2. Add 100 µL of each sample or control per well in duplicate or triplet. Controls and samples must be assayed at the same time.
3. Cover plate and incubate at 37°C for 1 hour.
4. Decant solution from the plate. Wash the wells 4 times with 300 µL wash buffer per well. Invert the plate and blot dry after the last wash.
5. Add 100 µL of the HRP-anti-Human IgA Fc Detection Antibody. Cover the plate and incubate at 37°C for 1 hour.
6. Repeat Step 4 as described.
7. Add 100µL one-step TMB to each well. Cover the plate and incubate at room temperature for 10 minutes, protected from light.
8. Add 50 µL stop solution to each well. The color in the wells should change from blue to yellow. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Within 30 minutes, read the optical density (O.D.) of all assay wells at 450nm in a microplate reader.

DATA ANALYSIS

Calculation and interpretation of assay results

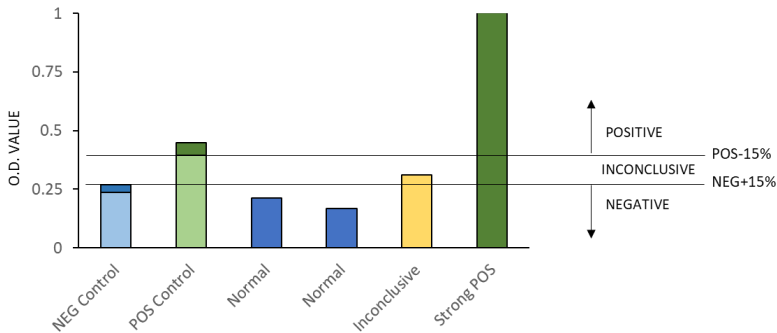
In order to evaluate the presence of Anti-2019-nCoV S1 Human IgA in a biological sample, the O.D. value of the sample must be compared to the values obtained for the positive and negative controls.

1. Calculate the average O.D. value of each control and sample.
2. Classify each sample as positive or negative based on the following:

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NEGATIVE: Sample O.D. value < 1.15 X O.D. of negative control
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.....
POSITIVE: Sample O.D. value > 0.85 X O.D. of positive control
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.....
INCONCLUSIVE: $1.15 \times \text{NEG} \leq \text{Sample O.D.} \leq 0.85 \times \text{O.D. POS}$
.....



Limitations:

1. The 2019-nCoV S1 Human IgA ELISA Kit is limited to the qualitative detection of antibodies specific for the SARS-CoV-2 virus.
2. A negative or non-reactive result can occur if the quantity of antibodies for the 2019-nCoV virus present in the specimen is below the detection limit of the assay.
3. This ELISA test kit is for research use only. This kit is not for use in diagnostic and therapeutic procedures. This kit is not validated for use in donor serum screening.

RESOURCES

References

1. Zhou P, et al: A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020, 579:270-89.
2. Xiao X, et al: The SARS-CoV S glycoprotein. *Cell Mol Life Sci*. 2004, 61 (19-20): 2428-30.
3. Long Q, et al: Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nature Medicine*. 2020, 29 April.
4. Ma H, et al: Serum IgA, IgM, and IgG responses in COVID-19. *Cell Mol Immunol*. 2020, 17: 773-775.

Related products

Catalog Number	Name	Species	Tag	Sequence
C19SD-G241H / F	2019-nCoV Spike protein RBD, HIS or Fc Tag	SARS-CoV-2	HIS / Fc	319-541
C19S1-G241H / F	2019-nCoV Spike protein S1, HIS or Fc Tag	SARS-CoV-2	HIS / Fc	16-685
C19S2-G241F	2019-nCoV Spike protein S2, Fc Tag	SARS-CoV-2	Fc	686-1212
C19S1-60DH	Anti-2019-nCoV Spike Protein hlgG Antibody	Human, IgG	HRP	Monoclonal
C19S1-61MH	Anti-2019-nCoV Spike Protein mlgG Antibody	Mouse, Fc		Monoclonal
C19S1-A60H	Anti-2019-nCoV Spike Protein hlgA Antibody	Human, IgA		Monoclonal
C19NP-60DH	Anti-2019-nCoV N-Protein hlgG Antibody	Human, IgG	HRP	Monoclonal
C19SD-876	2019-nCoV S1 Protein ELISA Kit			
C19S1-877	2019-nCoV S1 Human IgG ELISA Kit			
C19S1-P877	2019-nCoV S1 Human IgG-IgA-IgM ELISA Kit			
C19NS1-A877	2019-nCoV N-S1 Human IgA ELISA Kit			
C19NS1-P877	2019-nCoV N-S1 Human IgG-IgA-IgM ELISA Kit			