SignalChem

2019-nCoV S1 Protein ELISA Kit

Instruction Manual

Catalog No. C19SD-876

For the measurement of 2019-nCoV Spike Protein in complex biological samples

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.

For the detection of pathogens, RT-PCR method is the earliest method that can be applied. Real-time fluorescent RT-PCR positive is used as one of the confirmed diagnosis of new coronavirus infection. Due to the problems associated with the diagnosis of nucleic acid detection, such as false negatives, equipment and lab personnel expertise requirements and long detection time, additional CT scans and other clinical symptom indicators are needed for a proper diagnosis of COVID-19. The patient's serological indicators being positive for the specific IgM or IgG antibodies against 2019-nCoV emerges as the second diagnostic method. Antibody detection is based on the human immune response to foreign pathogens. If pathogen-specific IgM is detected in the serum, it indicates that infection has recently occurred. Usually after 20 days, specific IgG antibodies against pathogens can be detected. It can be used as an auxiliary diagnostic method when the nucleic acid test is negative. The FDA's 2019-nCoV testing guidelines have clearly stated that antibody testing cannot be used alone for the diagnosis of COVID-19, and cannot exclude infection or indicate the status of infection.

SignalChem 2019-nCoV S1 Protein ELISA kit provides an option to detect the pathogen at early stage of disease infection. The spike glycoprotein (S) of coronavirus belongs to the type I transmembrane protein, which contains two subunits, S1 and S2, which is also known to be the key component to bind with host cells through the interaction with angiotensin-converting enzyme 2 (ACE2). A receptor binding domain (RBD) of S1 can recognize the cell surface receptor and the mutation of RBD could cause higher motility rate. Though the coronavirus uses many different proteins to replicate and invade cells, the spike protein is the major surface protein that it uses to bind to a receptor — another protein that acts like a doorway into a human cell. This ELISA kit is designed to detect and measure nCoV Spike protein in complex biological samples such as saliva, pharyngeal swabs, serum or other samples.

About this assay

This 2019-nCoV S1 Protein ELISA Kit is a sandwich enzyme-linked immunosorbent assay for the quantitative measurement of 2019-nCoV Coronavirus Spike protein. The S1 Protein ELISA employs specific capture protein, ACE2, coated on a 96-well plate. Calibrators and samples are pipetted into the wells; the target protein in the calibrators and samples binds to the immobilized ACE2 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 protein bound.

GENERAL INFORMATION

Materials supplied

Part #	Part		Quantity / Size
C19S1-61DH	Anti-nCoV S1 Detection Antibody, 1X		1 vial x 10 mL
CP02-A51C2	ACE2 (19-740) Coated Plate		1 plate
CS02-506	One Step TMB Substrate		1 vial x 10 mL
E01-09	nCoV ELISA Sample Dilution Buffer		2 vials x 10 mL
SS01-09	Stop Solution		1 vial x 6 mL
C19SD-CAL0	Calibrators:	0 ng/ml	1 vial x 1 mL
C19SD-CAL1		5 ng/ml	1 vial x 1 mL
C19SD-CAL2		20 ng/ml	1 vial x 1 mL
C19SD-CAL3		80 ng/ml	1 vial x 1 mL
WB20-09	Wash Buffer, 20X		2 vials x 15 mL

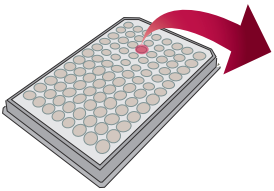
Additional materials required

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 and 540 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



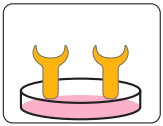
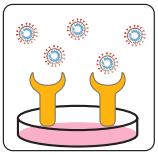
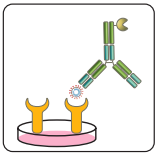


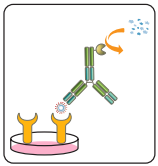
Plate pre-coated with ACE2



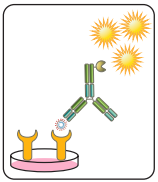
Add sample



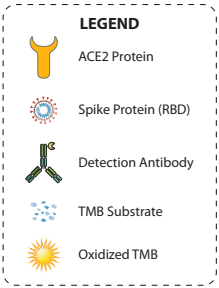
Wash plate & add detection antibody



Wash plate & add TMB substrate



Add stop solution & read plate



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 the 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 samples can be diluted with the nCoV ELISA Sample Dilution Buffer provided in the kit. The dilution required may be different depending on the property of the biological samples. It is recommended that several dilutions of samples be performed to determine the optimal concentration.

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 samples and calibrators per well in duplicate or triplicate.
3. Cover plate and incubate at 37°C for 1 hour.
4. Decant solution from the plate. Wash the wells 4 times with 300 uL wash buffer. Invert the plate and blot dry after last wash.
5. Add 100 µL of the HRP-anti-nCoV S1 Detection Antibody. Cover plate and incubate at 37°C for 1 hour.
6. Repeat Step 4 as described.
7. Add 100 µL one-step TMB to each well. Cover 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 and 540nm in a microplate reader.

DATA ANALYSIS

Calculation of Spike protein concentration

The 2019-nCoV Spike protein concentration of a biological sample may be calculated using the Calibrator by generating a standard curve that correlates the measured O.D. values.

1. Subtract the OD value measured at 540 nm from that measured at 450 nm for each assay well. This subtraction will correct for optical imperfections in the plates. Readings made directly at 450 nm without correction may be higher and less accurate.

2. Subtract the average OD value of the Calibrator 0 wells from the values of those wells that have samples or Calibrators.

3. Generate a standard curve by plotting the known Calibrator amounts (x-axis) versus O.D. value (Y-axis) as log10 values or using a logarithmic scale. Determine the linear regression equation for the data and ensure that the R^2 value is close to 1 (see Figure 1).

4. Using the OD value of a sample (after background subtraction) use the linear regression equation from the standard curve to calculate the concentration of the sample, ensuring that the sample OD is within the range of the standard curve. Correct for dilution to determine the actual concentration.

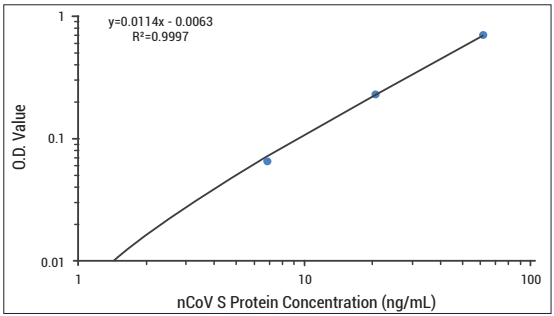


Figure 1. Typical calibration curve of the supplied Calibration standards. Data was analyzed using MS Excel® software.

In general, a good standard curve should have the following characteristics:

- R-squared value is greater than 0.95, and as close to 1 as possible.
- The OD of the Calibrator 0 should be lower than 0.25.
- The OD of the Calibrator 3 should be higher than 0.8.

Limitations:

1. The Calibrators provided in this kit are prepared by using recombinant 2019-nCoV Spike protein RBD in the sample dilution buffer, instead of native SARS-CoV-2 Spike protein. The real concentration of Spike protein will be different from the calculated concentration.

2. In native biological samples, there are various interfering substances that affect the accuracy of the test results. Thus, the results of this test can only be used for research purpose and not for diagnosis and therapy application.

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. Lan J, et al: Crystal structure of the 2019-nCov Spike receptor-binding domain bound with the ACE2 receptor. bioRxiv. doi: https://doi.org/10.1101/2020.02.19.956235.

Related products

Name	Catalog Number	Species	Tag	Expression System	Sequence
2019-nCoV Spike protein RBD	C19SD-G241H	Virus	HIS	CHO cells	319-541
2019-nCoV Spike protein RBD	C19SD-G241F	Virus	Fc	CHO cells	319-541
2019-nCoV Spike protein S1	C19S1-G241H	Virus	HIS	CHO cells	16-685
2019-nCoV Spike protein S1	C19S1-G241F	Virus	Fc	CHO cells	16-685
Anti-2019-nCoV Spike Protein	C19S1-61H	Human, IgG1		CHO cells	Monoclonal
2019-nCoV S1 Human IgG ELISA Kit	C19S1-877				