SignalChem

# 2019-nCoV S1 Human IgG ELISA Kit

## Instruction Manual

## Catalog No. C19S1-877

For the detection of Human IgG 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.*

# CONTENTS

# 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 anti-nCoV S1 Human IgG ELISA Kit is an enzyme-linked immunosorbent assay for the detection of Human anti-nCoV Spike protein S1 IgG in serum or other biological samples. Based on indirect ELISA principles, the anti-nCoV S1 Human IgG ELISA employs recombinant nCoV Spike protein coated on a 96-well plate. Controls and samples (with necessary dilution) are pipetted into the wells; the target IgG 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 IgG bound.

# GENERAL INFORMATION

## Materials supplied

| Part # | Part | Quantity / Size |
|---|---|---|
| H38-63DG | Anti-Human IgG Fab 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 IgG Sample Dilution Buffer | 2 vials x 10 mL |
| SS01-09 | Stop Solution | 1 vial x 6 mL |
| C19S1-NEG | Negative Control | 1 vial x 1 mL |
| C19S1-POS | Positive Control | 1 vial x 1 mL |
| WB20-09 | Wash Buffer, 20X | 2 vials x 15 mL |

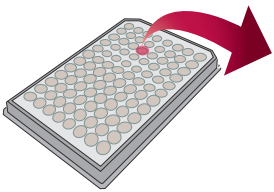
## Additional materials required

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 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



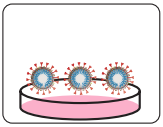
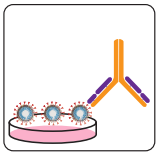
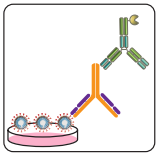


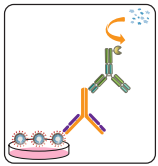
Plate pre-coated with
2019-nCoV Spike protein RBD



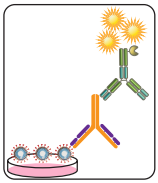
Add sample



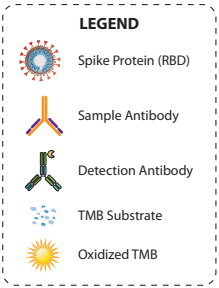
Wash plate & add detection antibody



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 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 IgG 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.

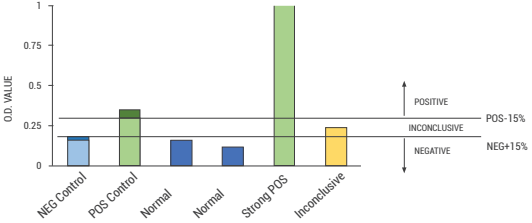
1. Place the required number of pre-coated strips onto an ELISA plate frame.
2. Add 100 µL of each sample or control per well in duplicate or triplet. Controls and samples must be assayed at the same time.
3. Cover plate and incubate at 37°C for 1 hour.
4. Decant solution from the plate. Wash the wells 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 IgG Fab Detection Antibody. Cover the plate and incubate at 37°C for 1 hour.
6. Repeat Step 4 as described.
7. Add 100uL 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 and 540nm in a microplate reader.

# DATA ANALYSIS

## Calculation and interpretation of assay results

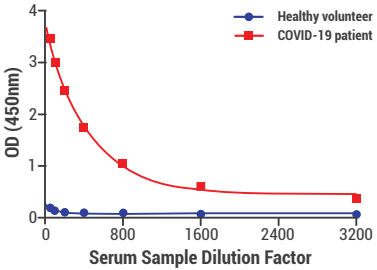
In order to evaluate the presence of Anti-2019-nCoV S1 Human IgG in a biological sample, the O.D. value of the sample must be compared to the values obtained for the positive and negative controls.

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




| | |
|---|---|
| NEGATIVE: | Sample O.D. value < 1.15 X O.D. of negative control |
| POSITIVE: | Sample O.D. value > 0.85 X O.D. of positive control |
| INCONCLUSIVE: | 1.15 X NEG ≤ Sample O.D. ≤0.85 X O.D. POS |

## Representative data



## Limitations:

1. The 2019-nCoV S1 Human IgG 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 Quan-Xin Long. Nature Medicine. 2020, 29 April.

## 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 Protein ELISA Kit | C19SD-876 | | | | |