# Human anti-2019 nCoV(N) IgG ELISA Kit

V1.0

**Catalogue No.**: FTEH4395

**Size**: 96T

**Reactivity**: Human

**Application**: This immunoassay kit allows for the qualitative determination of nCoV-IgG antibody in human serum, plasma, saliva & nasal fluid

**Storage**: 2-8℃ for 6 months.

**NOTE: FOR RESEARCH USE ONLY.**

## Kit Components

| Item | Specifications(96T) | Storage |
|---|---|---|
| Coated assay plate | 1vial | 2-8℃ |
| Negative Control (Ready-to-use) | 1vial | 2-8℃ |
| Positive Control (Ready-to-use) | 1vial | 2-8℃ |
| Sample Dilution Buffer | 1vial | 2-8℃ |
| HRP-conjugated anti-human IgG antibody (Concentrated) | 1vial | 2-8℃ |
| Antibody Dilution Buffer | 1vial | 2-8℃ |
| Wash Buffer (25 x concentrate) | 1vial | 2-8℃ |
| TMB Substrate | 1vial | 2-8℃ |
| Stop solution | 1vial | 2-8℃ |
| Adhesive Strip (For 96 wells) | 3 | 2-8℃ |
| Instruction manual | 1 copy | |

## Principle of the Assay

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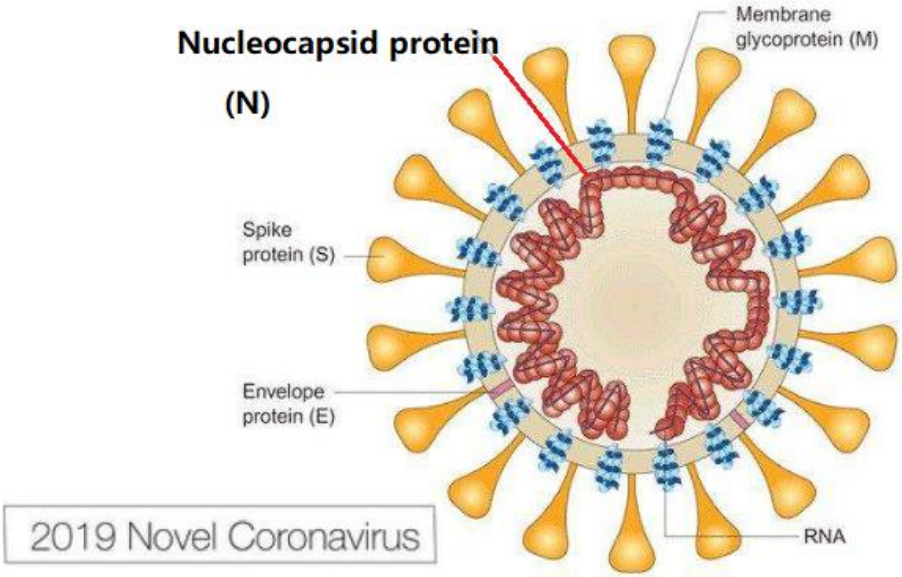
This kit was based on indirect enzyme-linked immune-sorbent assay technology. Recombined nCoV Nucleocapsid protein (antigen) was pre-coated onto 96-well plates. The test samples were added to the wells, unbound conjugates were washed away with wash buffer. Then added HRP conjugated anti-human IgG, if there were any nCoV(N) -IgG antibody in the samples, it would form a complex. TMB substrates were used to visualize HRP enzymatic reaction. It was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.




## Sequence of Nucleocapsid protein (antigen)

MSDNGPQNQR NAPRITFGGP SDSTGSNQNG ERSGARSKQR RPQGLPNNTA SWFTALTQHG KEDLKFPRGQ GVPINTNSSP DDQIGYYRRA TRRIRGGDGK MKDLSPRWYF YYLGTGPEAG LPYGANKDGI IWVATEGALN TPKDHIGTRN PANNAAIVLQ LPQGTTLPKG FYAEGSRGGS QASSRSSSRS RNSSRNSTPG SSRGTSPARM AGNGGDAALA LLLLDRLNQL ESKMSGKGQQ QQGQTVTKKS AAEASKKPRQ KRTATKAYNV TQAFGRRGPE QTQGNFGDQE LIRQGTDYKH WPQIAQFAPS ASAFFGMSRI GMEVTPSGTW LTYTGAIKLD DKDPNFKDQV ILLNKHIDAY KTFPPTEPKK DKKKKADETQ ALPQRQKKQQ TVTLLPAADL DDFSKQLQQS MSSADSTQA



**Wuhan Fine Biotech Co., Ltd.**

B9 Bld, High-Tech Medical Devices Park, No. 818 Gaoxin Ave.East Lake High-Tech Development Zone.Wuhan, Hubei, China(430206)

Tel :( 0086)027-87384275 Fax: (0086)027-87800889 www.fn-test.com

## Precautions for Use

1. After opening and before using, keep plate dry.
2. Before using the Kit, balance the reagents at room temperature at least 30 mins.
3. Storage TMB reagents avoid light.
4. Washing process is very important, not fully wash easily cause a false positive.
5. Don't let Micro plate dry at the assay, for dry plate will inactivate active components on plate.
6. Don't reuse tips and tubes to avoid cross contamination.
7. Avoid using the reagents from different batches together.

## Material Required But Not Supplied

1. Microplate reader (wavelength: 450nm)
2. 37℃ incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

## Manual Washing

Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of THREE washes.

## Automated Washing



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Aspirate all wells, then wash plate THREE times with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

## Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20℃ for long term. Avoid multiple freeze-thaw cycles.

- Serum: Coagulate the serum at room temperature (about 1 hour). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20℃.
- Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Analyze immediately or aliquot and store frozen at -20℃.
- Saliva & Nasal fluid: Centrifuge samples for 20 minutes at 10000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

**Note:** Samples to be used within 5 days may be stored at 2-8℃, otherwise samples must be stored at -20℃ (≤1 month) or -80℃ (≤2 months) to avoid loss of bioactivity and contamination.
Hemolyzed samples are not suitable for use in this assay.

## Wash Buffer Preparation:

Dilute 50mL of Concentrated Wash Buffer into 950 mL of Wash Buffer with deionized or distilled water.

## Preparation of HRP-conjugated human IgG Working Solution:

Prepare it within 1 hour before experiment.



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1） Calculate required total volume of the working solution: 50ul / well × quantity of wells. (Allow 55-60ul more than the total volume.)

2）Dilute the HRP-conjugated human IgG with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1µl HRP-conjugated human IgG into 99µl Antibody Dilution Buffer.)

## Assay Procedure

1. Bring all reagents to room temperature before use.
2. Label the sample wells, 2 Negative Controls, 2 Positive Controls and 1 blank well
3. Add 45 µL sample dilution buffer to each sample well.
   Add 50 µL sample dilution buffer for blank well.
4. Add 5µL sample to each sample well.
   Add 50ul Negative Controls and Positive Controls to set Controls well and gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37℃ for 30 min.
5. Remove the cover, and wash plate 5 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 min.
6. Add 50 µL HRP-conjugated human IgG to each well, except blank well
7. Seal the plate with a cover and incubate at 37℃ for 30 min.
8. Remove the cover, and wash plate 5 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 min.
9. Add 50 µl of TMB substrate into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 15-37℃ in dark within 10 min. And the shades of obvious blue can be seen in the Positive Controls. Blank well wells show no obvious color.
10. Add 50 µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
11. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution (Use the blank well to set zero).



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## Data Analysis

**Calculation of Results** (for reference only)

Cutoff Value (blank≤ 0.06， Negative Controls＜ 0.5， Positive Controls≥0.5)

## Sample test data

Rehabilitation clients from mobile cabin hospital. Plasma sample dilution 1:10, TMB Color development time 10 minutes at 18℃

| Rehabilitation clients(OD450) | | | | Healthy volunteers(OD450) | | | |
|---|---|---|---|---|---|---|---|
| 1# | 1.086 | 9# | 1.076 | 1# | 0.247 | 9# | 0.297 |
| 2# | 0.253 | 10# | 0.656 | 2# | 0.171 | 10# | 0.179 |
| 3# | 1.084 | 11# | 1.225 | 3# | 0.227 | 11# | 0.201 |
| 4# | 1.31 | 12# | 0.553 | 4# | 0.252 | 12# | 0.212 |
| 5# | 0.985 | 13# | 1.134 | 5# | 0.171 | 13# | 0.164 |
| 6# | 0.885 | 14# | 1.311 | 6# | 0.189 | 14# | 0.322 |
| 7# | 1.281 | 15# | 1.633 | 7# | 0.258 | 15# | 0.163 |
| 8# | 0.15 | 16# | 1.124 | 8# | 0.189 | blank | 0.048 |