Anti- 2019 nCoV(N) Ig ELISA Kit

V1.4

Catalogue No.: EU3125

Size: 96T

Reactivity: Universal

Application: This immunoassay kit allows for the qualitative determination of 2019-nCoV(N)-Ig antibody in serum, plasma and saliva and nasal fluid.

Storage: 2-8°C

Expiry Date: see kit label

Principle: Sandwich

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(96T)	Storage
Coated assay plate	1vial	2-8°C
Negative Control (Ready-to-use)	1vial	2-8°C
Positive Control (Ready-to-use)	1vial	2-8°C
Sample Dilution Buffer	1vial	2-8°C
Biotin-conjugated Nucleocapsid (Concentrated)	1vial	2-8°C(Avoid Direct Light)
Antigen Dilution Buffer	1vial	2-8°C
HRP-Streptavidin Conjugate(SABC)	1vial	2-8°C(Avoid Direct Light)
SABC Dilution Buffer	1vial	2-8°C
Wash Buffer (25 x concentrate)	1vial	2-8°C
TMB Substrate	1vial	2-8°C(Avoid Direct Light)
Stop solution	1vial	2-8°C
Plate Sealer	5pieces	
Product Description	1copy	

Wuhan Fine Biotech Co., Ltd.

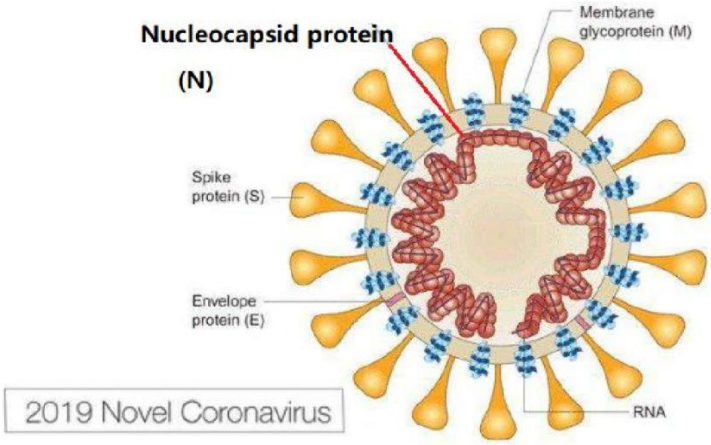
1st Floor, Building A02, Optics Valley Biomedical Industrial Park, Wuhan, China (430075)

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

Operation Procedure

Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Recombinant 2019-nCoV Nucleocapsid protein (antigen) was pre-coated onto 96-well plates. The Controls, test samples and Biotin-labeled antigen were added to the wells subsequently, and wash with wash buffer. HRP-Streptavidin Conjugate was added and unbound conjugates were washed away with wash buffer. TMB 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)

MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDLKFPRGQGVPINTNSSP
DDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGIIWVATEGALNTPKDHIGTRNPANNAAIVLQLP
QGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNSTPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQG
QTVTKKSAAEASKKPRQKRTATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEV
TPSGTWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLPAADLDDFSKQLQ
QSMSSADSTQA

Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.

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3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manufacturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Material Required but Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C 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

Washing

Manual: 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.

Automatic: Aspirate all wells, and then wash plate 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 shall be set for soaking 1 minute. (**Note:** set the height of the needles; be sure the fluid can be sipped up completely)

Sample Collection and Storage (universal)

- **Serum:** Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 minutes at approximately 1000×g, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
- **Plasma:** Collect plasma using (EDTA-Na_2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- **Saliva & Nasal fluid:** Centrifuge samples for 20 minutes at 1000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

Note: Samples to be used within 5 days can be stored at 2-8°C, besides that, samples must be stored at -20°C (assay ≤1 month) or -80°C(assay≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

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Reagent Preparation and Storage

Bring all reagents and samples to room temperature for 20 minutes before use.

1, Wash Buffer:

If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

Dilute 30ml Concentrated Wash Buffer into 750ml Wash Buffer with deionized or distilled water. Put unused solution back at 2-8°C.

2, Preparation of Biotin-labeled Antigen Working Solution:

Prepare it within 30minutes before experiment.

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 Biotin-labeled Antigen with Antigen Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul Biotin-labeled Antigen into 99ul Antigen Dilution Buffer.)

3, Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:

Prepare it within 30 minutes before experiment.

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 SABC with SABC Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul of SABC into 99ul of SABC Dilution Buffer.)

Assay Procedure

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test. .

1. Bring all reagents to room temperature before use.

2. Label the sample wells, 2 Negative Controls, 2 Positive Controls and 1 blank well. Adding sample and control (blank) wells!

3. Add 40ul sample dilution buffer to each sample well.

Add 50ul sample dilution buffer to blank well.

4. Add 10ul 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°Cfor 30 minutes.

5. Remove the cover, and wash plate 2 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 minutes.

6. Add 50ul Biotin-labeled Antigen to each well. Seal the plate with a cover and incubate at 37°C for 30 minutes.

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7. Remove the cover, and wash plate 3 times with Wash buffer and each time let the wash buffer stay in the wells for 1-2 minutes.

8. Add 50ul of SABC Working Solution into each well, cover the plate and incubate at 37°C for 30 minutes.

9. 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 minutes.

10. Add 50ul of TMB substrate into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in dark within 10-15minutes. And the shades of obvious blue can be seen in the Positive Controls. Blank well wells show no obvious color.

11. Add 50ul of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

12. 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).

Data Analysis

Calculation of Results

Cutoff Value =NCx× 2.1

NCx: Mean Absorbance of Negative Control (when NCx<0.05, Calculate as 0.05).

PCx: Mean Absorbance of Positive Control

1. Sample with absorbance values < Cutoff Value are considered negative.

Sample with absorbance value ≥ Cutoff Value are considered positive.

2. PCx≤0.5, the test is regarded as invalid, should be tested again.

Sample test data (for reference only)

Samples came from rehabilitation clients (1-2 months after recovery) of mobile cabin hospital. The plasma samples were diluted 1:5.TMB Color development time was 15 minutes at 37°C. NCx=0.109

Rehabilitation clients(OD450)				Healthy volunteers(OD450)			
1#	1.850	9#	1.876	1#	0.101	9#	0.112
3#	1.371	10#	1.556	2#	0.094	10#	0.095
3#	1.962	11#	2.010	3#	0.077	11#	0.088
4#	1.984	12#	1.458	4#	0.09	12#	0.099
5#	1.985	13#	1.957	5#	0.143	13#	0.134

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6#	1.885	14#	2.144	6#	0.128	14#	0.120
7#	2.046	15#	2.202	7#	0.112	15#	0.122
8#	1.295	16#	1.891	8#	0.096	blank	0.139

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