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Human Anti-2019 nCoV(N) IgM ELISA Kit

V1.1

Catalogue No.: EH4396

Size: 96T

Reactivity: Human

Application: This immunoassay kit allows for the qualitative determination of anti-nCoV-IgM in human serum, plasma, saliva and nasal fluid.

Storage: 2-8℃ for 6 months.

NOTE: FOR RESEARCH USE ONLY.

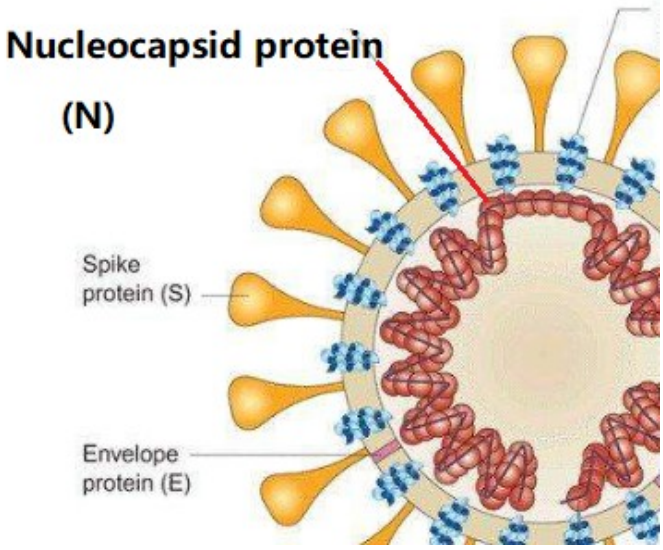
Kit Components

Item	Specifications(96T)	Storage
ELISA Microplate(Dismountable)	8×12	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 IgM 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℃

Plate Sealer	3	
Instruction manual	1 copy	

Principle of the Assay

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 IgM, if there were any anti-nCoV-IgM 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

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

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

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°C, 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:

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.

Preparation of HRP-conjugated anti-human IgM Working Solution:

Prepare it within 1 hour 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 HRP-conjugated anti-human IgM with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1µl HRP-conjugated anti-human IgM 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. Wash plate 2 times before adding sample and control (blank) wells!
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 3 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 anti-human IgM 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).

Data Analysis

Calculation of Results (for reference only)

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

Sample test data

Samples came from patients of mobile cabin hospital. The plasma samples were diluted 1:10. TMB Color development time was 10 minutes at 18℃.

Patients (OD450)				Healthy volunteers(OD450)			
1#	1.133	5#	1.630	1#	0.348	5#	0.394
2#	0.572	6#	1.108	2#	0.372	6#	0.319
3#	1.522	7#	0.965	3#	0.382	7#	0.266
4#	1.079	8#	0.977	4#	0.261	blank	0.057