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A Geno Technology, Inc. (USA) brand name

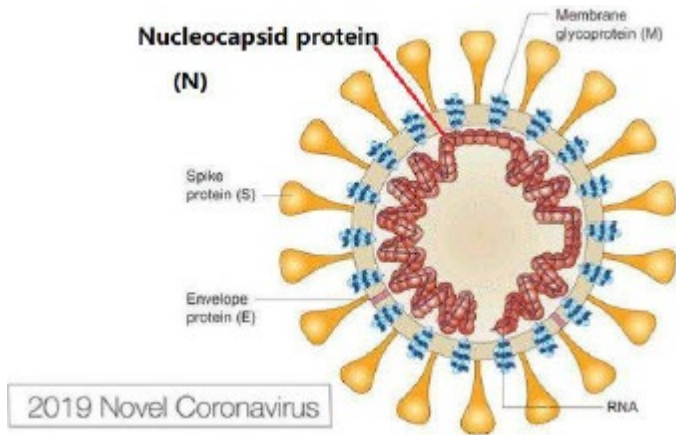
Human anti-SARS-CoV2 IgM ELISA Kit

**A Complete ELISA kit for the detection of SARS
CoV-2**

(Cat. #IT8430)

Introduction

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 anti-nCoV-IgG 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)

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MSDNGPQNR NAPRITFGGP SDSTGSNQNQ ERSGARSKQR RPQGLPNNTA SWFTALTQHG  KEDLKFRGQ GVPINTNSSP DDQIGYYRRA TRRIRGGDGK  
MKDLSRWYF YYLGTGPEAG  LPYGANKDGI IWVATEGALN TPKDHIGTRN PANNAIVLQ LPQGTTLPKG FYAEGSRGGS  QASSRSSRS RNSSRNSTPG  
SSRGTSPARM AGNGGDAALA LLLDRLNQL ESKMSGKGQQ  QQGQTVTKKS AAEASKKPRQ KRTATKAYNV TQAFGRRGPE QTQGNFGDQE LIRQGTDYKH  
WPQIAQFAPS ASAFFGMSRI GMEVTPSGTW LTYTGAIKLD DKDPNFKDQV ILLNKHIDAY  KTFPPTPKK DKKKKADETQ ALPQRQKKQQ TVTLLPAADL  
DDFSKQLQQS MSSADST
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ITEMS SUPPLIED

Item	Specification	Storage
ELISA Microplate	8x12	2-8C
Negative Control (ready to use)	1 vial	2-8C
Positive Control (ready to use)	1 vial	2-8C
Sample Dilution Buffer	1 vial	2-8C
HRP-conjugated anti-human IgG antibody	1 vial	2-8C
Antibody Dilution Buffer	1 vial	2-8C
Wash Buffer 25X	1 vial	2-8C
TMB Substrate	1 vial	2-8C
Stop Solution	1 vial	2-8C
Plate Sealer	3	
Instruction Manual	1 copy	

STORAGE CONDITIONS

The kit is shipped on blue ice. Upon arrival, store kit at 4°C for up to 6 months.

SPECIFICATIONS

- **Reactivity:** Human
- **Range:** 0.313-20ng/ml

- **Sensitivity:** 0.188ng/ml

ADDITIONAL ITEMS NEEDED

- Microplate reader (wavelength: 450nm)
- 37°C incubator
- Automated plate washer (Optional)
- Precision single and multi-channel pipette and disposable tips
- Clean tubes and Eppendorf tubes
- Deionized or distilled water

PRECAUTIONS

- We recommend performing pilot experiments using standards and a small number of samples.
- After opening and before using, keep plate dry.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- TMB Substrate must be protected from light.
- False positives may arise if washing steps are not completed.
- The use of duplicate well assays are recommended for both standard and sample testing.
- Do not let the plate dry out during the assay as this may inactivate active components.
- Do not reuse tips and tubes to avoid cross contamination.

PREPARATION BEFORE USE

Bring all reagents to room temperature before use.

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°C 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°C.

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°C.

Saliva & Nasal fluid: Centrifuge samples for 20 minutes at 10000xg 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°C (≤1 month) or -80°C (≤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 IgG 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 IgG with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1μl HRP-conjugated anti-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. Wash plate 2 times before adding sample and control (blank) wells!
3. Add 45 μ L sample dilution buffer to each sample well. Add 50 μ L sample dilution buffer for blank well.
4. Add 5 μ L sample to each sample well. Add 50 μ L 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°C 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 μ L HRP-conjugated anti-human IgG to each well, except blank well.
7. Seal the plate with a cover and incubate at 37°C 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 μ L of TMB substrate into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 15-37°C 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 μ L 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 \leq 0.06, Negative Controls $<$ 0.5, Positive Controls \geq 0.5)

Sample test data

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

Rehabilitation Patients (OD450)				Healthy Patients (OD450)			
1	1.086	9	1.076	1	0.247	9	0.297
2	0.253	10	0.656	2	0.171	10	0.197
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	0.25	16	1.125	8	0.189	Blank	0.048

RELATED PRODUCTS

Download our Protein Assay Development or Bioassay Handbook



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