Catalog Number: MBS3809907

96 Tests

Store all reagents at 2-8°C
Valid Period: 12 months
For samples: Serum

FOR RESEARCH USE ONLY! NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

1. INTENDED USE

This Human COVID-19 IgM antibody ELISA kit is a 2-hour ELISA for the detection of the COVID-19 IgM antibody of human serum. This ELISA kit for research use only, not for therapeutic or diagnostic applications!

2. INTRODUCTION

Coronaviruses are enveloped viruses with a positive-sense RNA genome and with a nucleocapsid of helical symmetry. Coronavirus nucleoproteins localize to the cytoplasm and the nucleolus, a subnuclear structure, in both virus-infected primary cells and in cells transfected with plasmids that express N protein. Coronavirus N protein is required for coronavirus RNA synthesis, and has RNA chaperone activity that may be involved in template switch. Nucleocapsid protein is a most abundant protein of coronavirus. During virion assembly, N protein binds to viral RNA and leads to formation of the helical nucleocapsid. Nucleocapsid protein is a highly immunogenic phosphoprotein also implicated in viral genome replication and in modulating cell signaling pathways. Because of the conservation of N protein sequence and its strong immunogenicity, the N protein of coronavirus is chosen as a diagnostic tool.

3. PRINCIPLE OF THE ASSAY

The human COVID-19 IgM antibody ELISA is made from the human IgM antibody coated microtiter plate, 2019-nCoV N protein-HRP and other reagents. It applies the capture ELISA principle to test the IgM against 2019-nCoV in human serum. In the test, the coated human IgM antibody combine with COVID-19 IgM antibody in serum, then add 2019-nCoV N protein -HRP to specifically bind with complex of antibody-IgM on the microplate. With the TMB substrate, it will generate an amount of color. The depth of color is relative with the content of the COVID-19 IgM antibody, when the value of color is greater than the cut-off value, the human has been infected with the 2019-nCoV.

4. MATERIALS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the label.

	MATERIALS	SPECIFICATION	QUANTITY
1	MICROTITER PLATE	96 wells	stripwell
2	CONJUGATE	11mL	1 vial
3	Negative control	1mL	1 vial
4	Positive control	1mL	1 vial
5	DILUENT(1X)	50mL	1 vial
6	SUBSTRATE A	6 mL	1 vial
7	SUBSTRATE B	6 mL	1 vial
8	STOP SOLUTION	6 mL	1 vial
9	WASH SOLUTION (20X)	50 mL	1 vial
10	INSTRUCTION	1	

5. MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

1) Precision pipettors and disposable tips to deliver 10-1000µl. A multi-channel pipette is desirable for large assays.
2) 100 mL and 1 liter graduated cylinders.
3) Distilled or deionized water.
4) Tubes to prepare sample dilutions.
5) Absorbent paper.
6) Microplate reader capable of measuring absorbance at 450 nm.
7) Centrifuge capable of 3000 × g.
8) Microplate washer or washing bottle.
9) Incubator (37°C).
10) Data analysis and graphing software.

6. SAMPLE PREPARATION

1) Sample dilution

Dilute sample with the sample diluent at 40 times. (5μl serum + 195μl diluent (1X)), the diluted sample need to mix evenly to get better results.

2) pH adjustment

High or low pH, detergents, urea, high salt concentrations, and organic solvents are some of the known interference factors. pH has a big impact on ELISA. So you must determine your sample pH and adjust it to 7.0-7.4.

NOTE:

1) The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.
2) The samples are human serum, which should be collected with no bacteria. The storage time should be less than 1 week at 2-8 Degrees Celsius, if for long term, it should be kept at -20 Degrees Celsius.
3) Avoid to use the samples with severe hemolysis, precipitate, contaminated by bacteria or protein suspension.
4) The EDTA, heparin sodium and other anticoagulants will not affect the results.

7. REAGENTS PREPARATION

1) Bring all kit components and samples to room temperature (20-25°C) before use.
2) Wash Solution - Dilute 50 mL of Wash Solution concentrate (100×) with 950 mL of deionized or distilled water to prepare 1000 mL of Wash Solution (1×). If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. The 1× wash solution is stable for 2 weeks at 2-8°C.
3) Do not dilute the other components which are ready- to-use.

8. ASSAY PROCEDURE

1) Secure the desired numbers of coated wells in the holder then add 100 μL of **Negative Control**、**Positive Control** or **Samples** to the appropriate well in the antibody pre-coated Microtiter Plate. Cover and incubate the plate for 45 min at 37°C.

2) Wash the microtiter plate using one of the specified methods indicated below:

a) Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with 1× wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of FIVE washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly

when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.

b) Automated Washing: Wash plate FIVE times with diluted wash solution (350-400µL/well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.

3) Add 100µL of **Conjugate** to each well. Mix well. Cover and incubate the plate for 45 min at 37°C.

4) Wash the microtiter plate five times as above.

5) Add 50µL **Substrate A** and 50µL **Substrate B** to each well including control wells, subsequently. Cover and incubate for 10-15 minutes at 37°C. (Avoid sunlight).

6) Add 50µL of **Stop Solution** to each well including control wells. Mix well.

7) Determine the Optical Density (O.D.) at 450 nm using a microplate reader immediately.

NOTE:

1) It is recommended that all controls and samples be run in duplicate. controls and samples must be assayed at the same time. Shake the plate after adding samples, standards or conjugates to the wells manually or with a vortex. But do not shake during the incubation step as this might result in higher backgrounds and worse precision.
2) Cover or cap all kit components and store at 2-8° C when not in use.
3) Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag with desiccants and store at 2-8°C to maintain plate integrity.
4) When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
5) Do not mix or interchange different reagent lots from various kit lots.
6) Do not use reagents after the kit expiration date.
7) Read absorbance immediately after adding the stop solution.
8) Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
9) Because TMB is light sensitive, avoid prolonged exposure to light. Also avoid contact between TMB and metal, otherwise color may develop.
10) It is normal that the conjugate might have a cloudy appearance. It is inconsequential to assay results. Re-suspend it with a vortex.

9. CALCULATION OF RESULTS

1) For the assay to be valid the following specifications must be met. The Positive control mean (PC: OD450) must be greater than 0.7, the Negative control mean must be less than 0.2.

2) Cut-Off Value (C.O.) = 0.2×PC: OD450. The presence or absence of IgM to 2019-nCoV is determined by calculating the sample to C.O. ratio.
3) If the S/C.O. ≥1, the sample is classified as POSITIVE for COVID-19 IgM antibody;
4) If the S/C.O. ＜1, the sample is classified as NEGATIVE for COVID-19 IgM antibody.

10. CERTIFICATE OF ANALYSIS

1) In the same lot CV%: 2.8, 4.7
2) Different lot CV%: 9.1, 7.5
3) Spike Recovery: 79.8-114.4%

Limitations

It is common and reasonable to validate the kit to check if the kit antibodies and assay procedure yield acceptable specificity, accuracy, and precision, especially when you rely exclusively on this assay to detect COVID-19 IgM antibody.

The 2019-nCoV N protein in the standard product is a recombinant protein expressed in E. coli.

The antibodies used in this assay are monoclonal antibodies produced by immunizing mouse with human IgM.

The positive control in this kit is human IgM of 2019-nCoV N protein.

This kit is not suitable for samples which contain sodium azide (NaN3). The sodium azide will affect the reactivity of HRP and result in the underestimation of the COVID-19 IgM antibody levels.

11. SAFETY NOTES

1) This kit contains small amount of 3, 3', 5, 5'-Tetramethylbenzidine (TMB) in Substrate B. TMB is non-carcinogenic but it is hhazardous in case of skin contact, eye contact, ingestion and inhalation. In case of contact, rinse affected area with plenty of water.
2) The Stop Solution provided with this kit is an acid solution. Avoid contact with eyes, skin, and clothing. Wear protective gloves, clothing, and face protection.
3) Care should be taken when handling the Standard because of the known and unknown effects of it.
4) Care should also be taken to avoid contact of skin or eyes with other kit reagents or specimens. In the case of contact, wash immediately with water.
5) Do not pipette by mouth.
6) Avoid generation of aerosols.
7) Waste must be disposed of in accordance with federal, state and local environmental control regulations.