SARS-CoV-2 IgM/IgG Detection Kit (Immunofluorescence-Based)
Catalog Number: 41A226

For the qualitative determination of human IgM and IgG antibody against SARS-CoV-2 virus respectively in human whole blood, serum or plasma

(This package insert must be read in its entirety before using this product)

INTENDED USE

SARS-CoV-2 IgM/IgG detection kit is a lateral flow immunoassay which uses europium (III) nanoparticles as the fluorescent marker for the detection and qualitative measurement of IgM and IgG antibodies against the nucleocapsid protein (NP) of SARS-CoV-2 virus in human blood.

The kit is only used as a supplementary test indicator for suspected cases with negative nucleic acid test results of SARS-CoV-2 virus, or used in conjugation with the nucleic acid test in diagnosis of suspected cases of coronavirus disease 19 (COVID-19). It cannot be used as a basis for diagnosis and exclusion of COVID-19, and it is not suitable for screening of general population.

The product is only applicable to medical institutions, and intended for use by professional person.

A positive test result requires further confirmation, and a negative test result cannot rule out the possibility of infection of SARS-CoV-2 virus.

The product is limited to clinical use and emergency storage during the COVID-19 outbreak and cannot be used as a routine in vitro diagnostic reagent for clinical use. The test results of this kit are for clinical reference only. It is recommended to conduct a comprehensive analysis based on the clinical manifestations of patients and other laboratory tests.

Laboratory testing of SARS-CoV-2 virus shall be carried out in accordance with local requirements for biosafety.

SUMMARY

The novel coronaviruses belong to the β genus, now officially named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which caused the outbreak of a coronavirus-associated acute respiratory disease called coronavirus disease 19 (COVID-19). COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

Nucleocapsid protein (NP) is the most abundant protein on the helical nucleocapsid of coronaviruses, which envelopes the entire genomic RNA. NP also interacts with other viral structural proteins to play important roles during host cell entry and virus particle assembly and release. Anti-NP antibodies have been shown to be the earliest and the most predominant antibodies detectable in patient's blood samples after coronavirus infection.

ASSAY PRINCIPLE

SARS-CoV-2 IgM/IgG detection kit is based on the principle of an immunochromatography in vitro test for the qualitative determination of antibodies against SARS-CoV-2 NP protein. When the sample is added to sample pad, it moves to the conjugate pad and resuspends NP-conjugated europium (III) nanoparticles that are dried on the conjugate pad. NP-conjugated europium (III) nanoparticles bind to

anti-NP antibodies in the specimen and form an antibody-NP- europium (III) nanoparticles complex. The mixture moves along the nitrocellulose membrane by capillary action and reacts with anti-human IgM as well as anti-human IgG antibodies that have been immobilized in the test reaction area separately. If antibody against SARS-CoV-2 is present enough in the sample, a fluorescent band in the test reaction area is appeared under UV. If there is no antibody against SARS-CoV-2 or not sufficient in the sample, the area will remain colorless. The sample continues to move to the control reaction area, and forms a fluorescent band, indicating the test is working properly and the result is valid.

SUPPLIED REAGENTS AND MATERIALS

A	Test Chip	50 T/Box
B	Sample diluent buffer	5ml/Bottle, X2
C	graduated pipette	50/Box

COMPOSITION OF REAGENTS

A. The Test Chip includes:

1. A nitrocellulose matrix test strip in which anti-human IgM antibody, anti-human IgG antibody and control protein have been immobilized on the two test lines and on the control line of strip, respectively.
2. A conjugation pad containing NP-conjugated europium (III) nanoparticles as well as anti-control protein antibody- conjugated europium (III) nanoparticles.
3. A sample pad for the loading of specimen.

B. The sample diluent buffer includes BSA as a stabilizer, and ProClin 300 as a preservative in PBS.

STORAGE AND PREPARATION OF TEST SAMPLES

- Whole blood samples are suggested to be assayed immediately after collection.
- EDTA or heparin are recommended as anticoagulants to collect plasma samples. The concentration of EDTA or heparin should be below 800 μg/ml.
- Serum or samples are suggested to be assayed immediately after separation, or preferably stored frozen (-20^{0}C or below) for less than 1 month in aliquots. Multiple freeze-thaw cycles should be avoided. Specimens are suggested to be balance to room temperature (18^{0}C -28^{0}C) before detection.
- When required, vortex test serum or plasma samples at room temperature to ensure homogeneity. Then centrifuge samples at 10,000 to 15,000 rpm for 5 minutes prior to assay to remove particulates. Please do not omit this centrifugation step if samples are cloudy and containing particles.
- Serum or plasma specimens with EDTA, sodium citrate or heparin can be tested.
- Hemolytic specimens as well as specimens with visible microbial contamination should not be used. Highly lipaemic or icteric specimens are not recommended.

STORAGE AND STABILITY

- Store as packaged in the sealed pouch at 4-30 °C, avoid hot and sunshine, valid for 6 months.
- Do not remove the Test Chip from the pouch until ready to use. The Test Chip should be used immediately once opened.

PRECAUTIONS AND SAFETY

1. For *In Vitro* Diagnostic Use.
2. Carefully follow the instructions and procedures described in this insert.
3. Tests should be applied by trained staff working in the laboratories where the sample(s) is taken by qualified medical personnel.
4. Neither inter-change materials from different product lots nor use beyond the expiration date. The use of medical device beyond expiration date will affect test result.
5. The Test Chip should remain in its original sealed pouch until ready to use. Do not use the Test Chip if the pouch is damaged or the seal is broken. Discard after single use.
6. Blood specimens, used test chips, graduated pipette and sample vials are potentially infectious. Proper laboratory safety techniques, handling and disposal methods should be followed in accordance with standard procedures and relevant regulations observed by microbiological hazard materials.
7. The graduated pipette should be used for one specimen only. Discard after single use.
8. Do not smoke, eat, or drink in areas in which specimens or kit reagents are handled.

ASSAY PROCEDURES

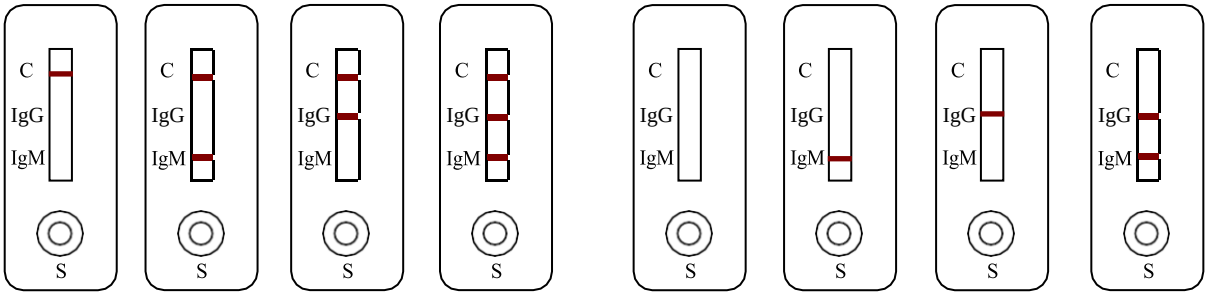
Please equilibrate the test chip as well as the sample diluent buffer to room temperature (20-25°C) for at least 30 minutes before use.

Step 1	Remove the Test Chip from the sealed pouch and place it on a clean and flat place.
Step 2	Use a micropipettor or a graduated pipette to add 10 µL (one drop by using graduated pipette) of serum, plasma or whole blood into the Sample Well (S) of Test Chip
Step 3	Add 2 drops (80-100µL) of sample diluent buffer by using the drop bottle provided in the kit into the Sample Well (S) of Test Chip immediately after adding specimen
Step 4	Observe the Result Window under UV and interpret the test result at 5-10 min using U2000i detection machine. (Note: Do NOT exceed 15 min.)

RESULTS

1. A fluorescent band will appear at the control reaction area (C) of the result window to show that the test is working properly.
2. Fluorescent bands that appeared at the IgM and/or IgG reaction area (IgM, IgG) of the result window 5-10 minutes after adding the sample diluent buffer indicates the test result. The fluorescent bands that appeared 15 minutes later are invalid.

INTERPRETATIONS OF THE RESULTS



Negative Result: The presence of only one band at the control reaction area (C) indicates a negative result. Negative result indicates that no SARS-CoV-2 NP antibodies have been detected with ImmunoDiagnostics SARS-CoV-2 IgM/IgG detection kit, therefore no serological indication of COVID-19 currently or in the past.

Positive Result:

1. IgM positive: In addition to the band in the control reaction zone (C), another fluorescent band with peak area > 8000 appears in the lower test reaction area (IgM).

2. IgG positive: In addition to the band in the control reaction zone (C), another band with peak area > 8000 appears in the middle test reaction area (IgG). Positive result indicates that SARS-CoV-2 NP IgG antibodies have probably been detected using ImmunoDiagnostics SARS-CoV-2 IgM/IgG detection kit

3. IgM and IgG both positive: In addition to the band in the control reaction zone (C), another two bands with peak area > 8000 appear. in the test reaction area (IgM and IgG). It indicates that SARS-CoV-2 NP IgM and IgG antibodies have probably been detected using ImmunoDiagnostics SARS-CoV-2 IgM/IgG detection kit

- Positive result indicates that SARS-CoV-2 NP IgM and/or IgG antibodies have probably been detected using ImmunoDiagnostics SARS-CoV-2 IgM/IgG detection kit.

- Samples with positive results should be retested using ImmunoDiagnostics SARS-CoV-2 IgM/IgG detection kit before data interpretation. Only repeatable positive results in the same sample can be finally considered as the successful detection of antibodies to SARS-CoV-2 NP, and can be used as serological indications of COVID-19 currently or in the past.

- If negative results show up during the repeated test, the previous positive results are false positive and these samples should be considered as negative. For more information regarding troubleshooting, please contact ImmunoDiagnostics' tech support.

Invalid Result: No band appears in the control reaction area after performing the test. In this case, repeat the test with a new Test Chip.

FOLLOW-UP ACTION

As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluate.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and specificity

A total of 46 cases of clinical samples (26 positive and 20 negative) were tested in the clinical evaluation. The positive samples were collected from COVID-19 confirmed cases with clinical symptoms, laboratory abnormalities (nucleic acid test) or pulmonary imaging manifestations.

Test Kit IgM	Percentage	Test Kit IgG	Percentage
Sensitivity	85%	**Sensitivity**	73%
Specificity	100%	**Specificity**	100%

2. Repeatability

Samples	Positive Rate of Test Chips
Borderline SARS-Cov-2 IgM Positive Sample	100% (10/10)
Borderline SARS-Cov-2 IgG Positive Sample	100% (10/10)
Negative control 1	0% (0/10)
Negative control 2	0% (0/10)

3. Reproducibility

Positive Samples	Positive Rate of Test Chips from Different Lots		
	Lot #1	Lot #2	Lot #3
#1	100% (10/10)	100% (10/10)	100% (10/10)
#2	100% (10/10)	100% (10/10)	100% (10/10)
#3	100% (10/10)	100% (10/10)	100% (10/10)
#4	100% (10/10)	100% (10/10)	100% (10/10)

4. Cross-Reactivity

Other common causative agents of infectious diseases were evaluated for cross reactivity. Some positive specimens of other common infectious diseases were spiked into the Novel coronavirus positive and negative specimens and tested a separately. No cross reactivity was observed with specimens from patients infected with HIV, HAV, HBsAg, HCV, TP, HTLV, CMV, FLUA, FLUB, RSV, MP, CP, HPTVs.

5. Interferences

The test result of SARS-CoV-2 IgM/IgG detection kit isn't interfered with the substances at the following concentration:

Analytes	Concentration	Analytes	Concentration
Albumin	20 mg/ml	Acetoacetic Acid	200 μg/ml
Bilirubin	20 μg/ml	Benzoylecgonine	100 μg/ml
Hemoglobin	15 mg/ml	Caffeine	200 μg/ml
Glucose	20 mg/ml	Ethanol	1.0%
Uric Acid	200 μg/ml	Gentisic Acid	200 μg/ml
Lipids	20 mg/ml	B-Hydroxybutyrate	20 mg/ml
Acetaminophen	200 μg/ml	Methanol	1.0%
Acetylsalicylic Acid	200 μg/ml	Heparin	800 μg/ml
EDTA	800 μg/ml	Phenothiazine	200 μg/ml
Phenylpropanolamine	200 μg/ml	Salicylic Acid	200 μg/ml

6. Limit of detection

A certain amount of clinical positive samples were diluted in different multiples until the concentration results at two intervals were negative and positive respectively. Then, the titer that can be steadily detected for 19 times in 20 tests was carefully confirmed within this concentration range, which is the titer of detection limit. 5 out of 10 positive samples were shown to be positive after 10^3- fold dilution. 2 out of 10 positive samples were shown to be positive after 10^4 –fold dilution. The Limit of Detection (LOD) is 10^4-fold dilution.

LIMITATIONS

1. Positive results should be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immune-suppressed individuals. Therefore, negative results obtained with ImmunoDiagnostics SARS-CoV-2 IgM/IgG detection kit are only indication that the specimen does not contain detectable level of antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with the virus.
3. The false positive results include cross-reactions with some components of blood from individual to NP protein. It has been observed that the hemolytic specimens lead to false positive results. In the case of false negative results, the most common factors are: non-responsiveness of antibodies to the NP protein by that certain unknown components are masking its epitope, such that antigen cannot be seen by the antibodies; and degraded other test components.
4. The effectiveness of the test is highly dependent on storage of kits and sample specimens at optimal conditions. The most common assay mistakes are using kits beyond the expiry date, contaminated reagents, incorrect assay procedure steps, failure to add specimens or reagents, timing errors, the use of highly hemolytic specimens, incompletely clotted serum specimens and additional substances in blood specimens. For more information, please contact ImmunoDiagnostics technical support for assistance.
5. The prevalence of the marker will affect the assay's predictive values.
6. This assay cannot be utilized to test pooled (mixed) serum or plasma. The kit has been evaluated only with individual serum or plasma specimens.
7. ImmunoDiagnostics SARS-CoV-2 IgM/IgG detection kit is a qualitative assay and the results cannot be used to measure antibody concentration.

SYMBOLS

	Manufacturer		Consult Instruction
	Expiry date		Store
	Lot number		Caution
	Catalog number		

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