dAb ImmunoTech
COVID-19 (SARS-CoV-2) IgG/IgM Dual Antibody Test

(Immunochromatographic Assay)

For Emergency Use Authorization (EUA) use only

COVID-19 IgG/IgM Dual Antibody Test

Instruction For Use

Symbol and Indication

REF	Catalog #	**Kit-LFI-001-25, Kit- LFI-001-50, Kit- LFI-001-100, Kit- LFI-001-B, Kit- LFI-001-O**
IVD	For in vitro diagnostic use only	

Document Overview

○ INTENDED USE

The dAb ImmunoTech COVID-19 (SARS-CoV-2) IgG and IgM Dual Antibody Test Kit (Product name: COVID-19 Ab-Test) is a single use qualitative Lateral Flow ImmunoAssay (LFIA) test that detects and differentiates both circulating IgM and IgG antibodies to SARS-CoV-2 in whole blood, serum, or plasma specimens from individuals with signs and symptoms of infection who are suspected of COVID-19 by a healthcare provider. The COVID-19 Ab-Test is an aid in the diagnosis of patients with suspected SARS-CoV-2 infection in conjunction with clinical presentation and the results of other laboratory tests. Results from the COVID-19 Ab-Test should not be used as the sole basis for diagnosis.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. 263a, to perform moderate and high complexity tests.

Results are for the detection of the two SARS-CoV-2 antibodies IgM and IgG. IgM antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although levels over the course of infection are not well characterized. IgG antibodies to SARS-CoV-2 become detectable later following infection. The IgG antibody is the most abundantly found immunoglobulin to be produced in response to a COVID-19 antigen. It will remain in the body after initial exposure for a long-term response period. Positive results for both IgG and IgM can occur after infection and can be indicative of acute or recent infection. All results including positives, negatives, false positives, and false negatives can be interpreted from the results. (see Figure 2, and Table 4)

○ BACKGROUND

Coronaviruses (CoV) are a family of single-stranded positive-sense RNA viruses that infect animals and humans. Several known coronaviruses are circulating in animals that have not yet infected humans. These are classified into 4 general categories based on their host specificity. There are seven known types of CoVs. Coronavirus has caused two large-scale pandemics in the last two decades, SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome) outbreaks in 2002 and 2012 respectively occurred when the virus crossed-over from animals to humans causing significant mortality.

More recently, Coronavirus disease 2019 (COVID-19) is defined as illness caused by a novel coronavirus now called Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2; formerly called 2019-nCoV). This virus was first identified amid an outbreak of respiratory illness cases in Wuhan City, Hubei Province, China. It was initially reported to the WHO on December 31, 2019. On January 30, 2020, WHO declared the COVID-19 outbreak a global health emergency. On March 11, 2020, WHO declared COVID-19 a global pandemic, its most recent pandemic declaration since declaring H1N1 influenza a pandemic in 2009. Patients with SARS-CoV-2 report a mild to severe respiratory illness with viral pneumonia. Clinical manifestations are fever, fatigue, and cough, which can rapidly develop into severe pneumonia, respiratory failure, septic shock, multiple organ failure, and severe acid-base metabolism disorders. COVID-19 is life-threatening and deadly infectious disease. Tests to detect the presence of the SARS-CoV-2 antibodies are a necessity, both on a national and international scale, to gauge what proportion of the population might be immune or be an asymptomatic carrier. Therefore, to manage the ongoing pandemic there is an urgent need for devices that can provide easy to use point-of-care, accurate and rapid antibody test results.

○ TEST PRINCIPLE

The dAb ImmunoTech COVID19 IgG/IgM Dual Antibody Test Kit is based on the principle of Lateral Flow ImmunoAssay (LFIA), also known as the Immunochromatographic Assay or strip test, is designed to detect the SARS-CoV-2 IgG and IgM antibodies in human serum, plasma and whole blood specimens. As experimentally validated, when the specimen is added to the specimen well the specimen is absorbed into the device by capillary action. If the specimen contains SARS-CoV-2 IgM and IgG antibodies, both antibodies will specifically bind to the colloidal gold-labeled SARS-CoV-2 antigens, respectively. The forming antibody/antigen complex will be captured by the mouse anti-human IgM and IgG secondary antibodies pre-immobilized on the nitrocellulose membrane. The signal lines can be seen on membrane when forming the complex as shown in following illustration (Fig.2). Finally, a red color will appear on both the IgM and IgG lines and indicate positive results. If neither antibody is present, no color will appear on Test Lines 1 and 2 and a negative test results. The internal Control line (C) in this test should always exhibit a clear red color to ensure that the test procedure is valid (Fig.1).

As internal quality control C line in this test, it always should exhibit clear red color, and ensure that the test throughout whole procedure is used correctly and result is valid (Fig.2).

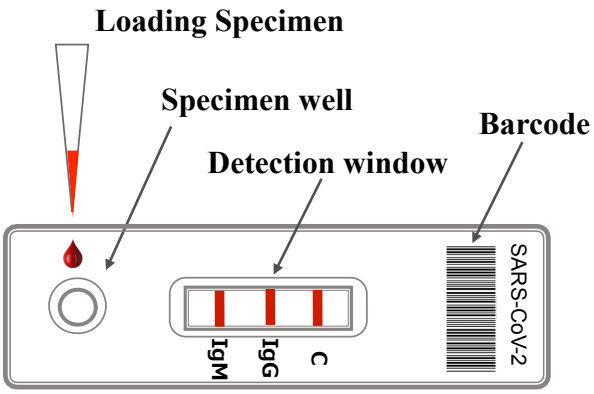


Fig.1. Illustration of Lateral Flow Immunoassay (LFI)

- **REAGENTS AND MATERIALS**
  - **Reagents and Materials Provided**

A different kit sizes and their components are provided and listed below:

Table 1. List of Components in Test Kit

NO	Components	Cat#/Kit Size (No of Tests)		
		Kit-LFI-001-25	Kit-LFI-001-50	Kit-LFI-001-100
1	Test Cassette	25	50	100
2	Assay Buffer	1 Bottle	1 Bottle	1 Bottle
3	Sterile Lancet Cap	25	50	100
4	Dropper	25	50	100
5	Swab	25	50	100
6	Instruction for use	1	1	1

- **Composition**

The dAb ImmunoTech COVID19 IgG/IgM Dual Antibody Test Kit contains the material compositions as listed in the Table 2 below.

Table 2. List of Compositions in Test Kit		
NO	**Compositions**	**Description**
1	Colloidal gold-conjugated novel coronoavirus antigen on the membrane	Conjugate Pad
2	Colloidal gold-labeled rabbit IgG	C Line
3	Goat anti-rabbit IgG antibody	The same above
4	Mouse anti-human IgG monoclonal antibody	G Line
5	Mouse anti-human IgM monoclonal antibody	M Line
6	Sample Buffer	0.01M PBS; PH 7.4

- **Other Material Required But Not Provided**

  Timer / Gloves

- **STORAGE AND STABILITY**

1. The kit should be stored at room temperature (2°C~30°C or 35.6°F~86°F) and not over 30°C. Under these conditions, the shelf-life is up to 18 months. Do not freeze the kit.
2. Keep the kit sealed and avoid to storing it under direct sunlight.
3. The test cassette must be used within 2 hours after opening. Never freeze the test cassette. The Specimen diluent should be used within 14 days after opening, and should be stored at 2°C~30°C or 35.6°F~86°F.

- **SPECIMEN COLLECTION AND PREPARATION**

The dAb ImmunoTech COVID19 IgG/IgM Dual Antibody Test Kit is suitable for testing the following type of specimens, including human whole blood, plasma and serum as shown in the Table 3. Most important, the specimens are collected and

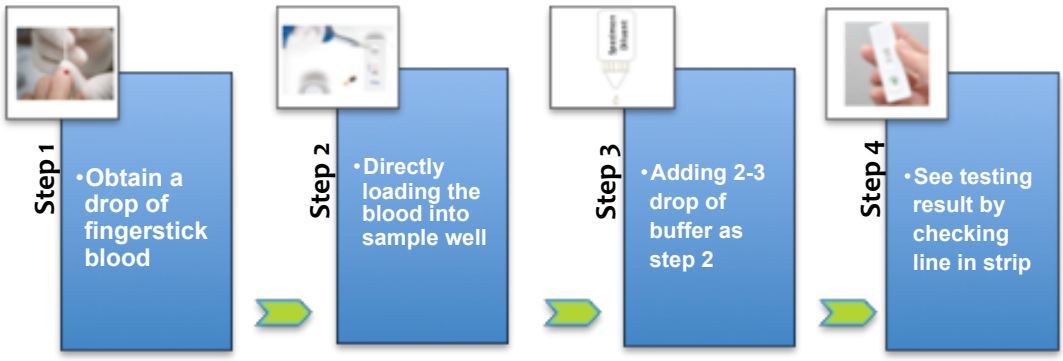
prepared by using standard biosafety procedures as described for all infectious materials of human origin.

Table 3. Sample storage and Preparation for COVID-19 IgG/IgM Dual Antibody Test Kit

Specimen	Description	Stability	Storage
Whole Blood	1. Take one drop of whole blood by using fingerstick. The blood specimen of whole can be prepared by using anticoagulant sample buffer. 2. The blood specimen has been tested in the kit. The specimen must be tested within 24 hours of collection.	7 days	At 2-8˚C
Plasma	1. Collect blood specimen into a blue top collection tube containing anticoagulants by venipuncture. 2. Separate the plasma by centrifugation. 3. Carefully withdraw the plasma into a new pre-labeled tube.	The same above	At 2-8˚C
		1 year	Minus -20°C
Serum	1. As above, collect blood specimen into a red top collection tube containing no anticoagulants buffer by venipuncture. 2. Allow the blood to clot. 3. Separate the serum by centrifugation. 4. Carefully withdraw the serum into a new pre-labeled tube.	The same above	At 2-8˚C
		1 year	Minus -20°C
General Requirements	For all frozen samples above, avoid more than 3 Freeze-Thaw cycles. Before testing, samples stored in refrigerated or frozen storage should be slowly returned to room temperature and stirred. When particulates are clearly visible in the sample, the precipitate should be removed by centrifugation before testing.	1 year	Minus -20°C

○ TEST PROCEDURE

As shown in following schematic illustration, there are four simple and straightforward steps for doing the test.



Schematic illustration of testing procedure for COVID19 IgG/IgM Dual Antibody Kit

- Step 1. Before use, check the Test Card to insure the foil pouch is undamaged and the Specimen Diluent is no leakage. Bring the Test cassette and the Specimen Diluent to room temperature before testing. The Test Card should be used within 2 hours after remove from foil pouch. The test kit is now ready for use. Using the enclosed capillary sampler, obtain 20µL of fingerstick whole blood specimen (See also Table 3 above).
- Step 2. Apply 10µL of serum or plasma or whole blood specimen and load to the specimen well of Test cassette.
- Step 3. Add 2 drops (80 µL) of Specimen Diluent to the specimen well, avoid air bubbles.
- Step 4. Wait for 10 minutes and read the results by viewing the detection window (See also Fig.1 and Fig.2). All wastes should be treated as potential infectious materials.

○ QUALITY CONTROL

The whole workflow is shown in Fig.1 and Test Procedure above. The quality of kit is controlled by a built-in quality control C line or called as Procedural Control, which is used to determine validity of the assay. The control C line must be

reactive (a definite red line) on each Stripe for the results of that Stripe to be valid. Here are a typical scenarios for control results below (Fig.2).

1. If the C line develops red color, it validates the test operation.
2. If the C line does not show a red color, the test is invalid, no matter what the Test Lines display. Lack of red color on the Control Line (C) indicates that the operation may not have been followed correctly or the kit is deteriorated. A new specimen and new test kit is required.

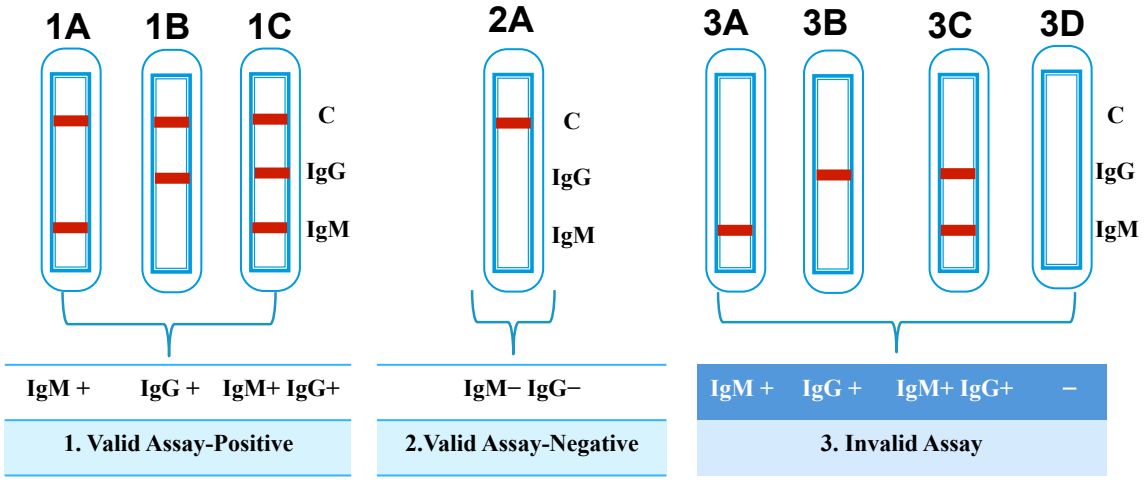


Fig.2. The three type of typical results are shown throughout assay

○ INTERPRETATION OF ASSAY RESULT

All the results are summarized and concluded in Table 4 and shown in Figure 2 in multiple and comprehensive parameters, such as visible data, result analysis, solution, clinical findings and etc.

Table 4. Summary for interpretation of assay result

Item	Valid Assay -Typical Scenarios							Invalid Assay (3A-3D)
	IgM positive (1A)	IgG positive (1B)	IgG and IgM Positive(1C)	IgG weak positive (4A)	IgM weak positive (4B)	IgG and IgM weak Positive (4C)	Negative (2A)	
Indication	C Line + G line – M Line +	C Line + G line + M Line –	C Line + G Line + M Line +	C Line + G line + M Line –	C Line + G line – M Line +	C Line + G Line + M Line +	C Line + G line – M Line –	C Line – G Line +/– M Line +/–
Rusult Analysis	Acceptable ✔	Acceptable ✔	Acceptable ✔	Acceptable ✔	Acceptable ✔	Acceptable ✔	Acceptable ✔	If the C Line does not develop, the assay is invalid regardless of color development of the G or M Lines.
	With an acute or recent infection	With recent or previous infection	With current or recent previous infection	With possible current or recent previous infection	The same left	The same left	With no current or recent previous infection	
Solution	Pass	Pass	Pass	Very cautious and also check with other methods, liking RT-PCR.			Particularly for patients who have been in contact with known infected persons or in areas with high prevalence of active infection	Not Acceptable, also Repeat the assay with a new kit
Conclusion	1. Not as the sole basis to diagnose or exclude SARS-CoV-2 infection. 2. False positive results may occur due to cross-reacting antibodies from previous infections. 3. Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a diagnostic determination is made.						Confirm by other assay	Also indicated (3A to 3D) in Fig.2
Legend Key		Positive +		Weak Positive +		Negative –		

- **PERFORMANCE CHARACTERISTICS**

1. Clinical Performance

In order to entirely evaluate the dAb ImmunoTech COVID19 IgG/IgM Dual Antibody Test Kit, blood samples were collected and prepared from SARS-CoV-2-infected patients and healthy individuals from multiple hospitals and CDC laboratories in China and USA. A total of 206 samples were tested. The samples were 59 clinically and RT-PCR test confirmed SARS-CoV-2 positive, and 147 of non-SARS-CoV-2-infected negative people. All tested samples were inactivated before used. The tests were done separately at each site. The tested results of all samples by our own developed Kit were summarized and analyzed in the Table 5 below:

Table 5. Evaluation and Validation of Test Kit (dAb ImmunoTech COVID-19 IgG/IgM Dual Antibody Test Kit)

Typical Tested Data			Comparater/Clinical truth							
			Sample Tested Result			Specificity	Sensitivity	Cross Reactivity	Stability	Concordance Rate
			Positive Sample	Negative Sample	Subtotal					
			59	147	206					
Assay Parameters	Positive Sample	C Line + G Line – M Line +	10	1	11	98.64% (Overall) (95% CI: 94.67%~99.76%)	93.22% (Overall) (95% CI:82.73% ~97.81%)	No any reacted with tested a variety of molecules , liking antigens and antibodies (See Section 6.4)	Kit still has activity and also keeps stable until last test by May,2020. It will be checked again in different time periods, such as after one year storage	93% (Overall)
		C Line + G Line + M Line–	5	1	6					
		C Line + G Line + M Line +	40	0	40					
	Negative Sample	C Line + M line – G Line –	4	145	149					
Legend Key	Positive +	§ The tested all 59 of positive samples collected from patients who confirmed positive with a RT- PCR method for SARS-CoV-2 infection and also came with mild or no clinical symptoms.								
	Negative –	§ The tested all 147 of negative samples collected from healthy individuals without clinical symptoms before SARS-CoV-2 infectious outbreak in December of 2019.								

Percent Positive Agreement: 93.22%, Negative Percent Agreement:98.6%

2. Assay Cross Reactivity and Interference

Cross-Reactivity and Interference of the dAb ImmunoTech COVID19 IgG/IgM Dual Antibody Test with all tested molecules were evaluated using serum or plasma samples which contained antibodies to the pathogens and antigen. A total of 17 related and unrelated molecules were applied and checked for across reactivity and interference of compositions in the kit. 13 antibodies, 1 antigen, and 3 blood-enriched proteins were used separately and respectively.

No false positives or false negatives were reported.

- **WARNINGS AND PRECAUTIONS**
  - This package insert must be read completely before performing the test. Failure to follow the insert may give inaccurate test results. This kit has been approved for use with serum and plasma specimens only.
  - Use of this test kit with specimens other than those specifically approved for use with this test kit may result in inaccurate test results.
  - Users of this test should follow the CDC Universal Precautions for prevention of the transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens.

See general and special considerations in Table 6 below:

Table 6. Warnings and Precautions When Using Kit

No	Do's	Don'ts
1	This package insert must be read completely before performing the test	Do not use test cassette, buffer solution, or any kit component beyond the indicated expiration date.
2	Once opened, the cassettes should be used within 2 hours.	Do not use test cassette, buffer solution, or any other kit components if the pouch is damaged or the seal is broken.
3	Handle specimens in accordance to the OSHA Standard on Bloodborne Pathogens	Do not immerse test cassette in water.
4	Dispose of all used or damaged test cassettes, capillary samplers, or other kit component as biohazardous materials.	Do not use samples containing lipids, hemolysis, or turbidity which can affect results.
5	Wash hands thoroughly after handling specimens and testing.	Do not freeze test cassette or buffer solution.

LIMITATIONS OF THE KIT

1. The kit is designed to test only human whole blood, plasma, and serum specimens. It is not suitable for testing other types of human specimens.
2. The kit is a qualitative assay and cannot determine the quantitative concentration of SARS-CoV-2 antibody in the specimens above. Therefore, the intensity of the test line does not necessarily correlate to SARS-CoV-2 antibody titer and concentration in the specimens.
3. A positive result is only for clinical reference and key considerations for SARS-CoV-2 infection, and should not be the only basis for diagnosis. A confirmed diagnosis should only be made after combining with all other clinical findings and other additional laboratory testing and evaluations.
4. The Assay Procedure and the Test Result Appearance and Interpretation must be followed closely when testing for the presence of IgG and IgM antibodies to SARS-CoV-2 in blood, plasma or serum from individual subjects. Failure to follow the procedure may give inaccurate results.
5. Nonreactive results can occur if the quantity of SARS-CoV-2 IgG and IgM antibodies present in the sample is below the detection limits of the assay, or if the SARS-CoV-2 IgG and IgM antibodies detected are not present during the stage of disease in which a sample is collected.
6. A nonreactive result for an individual subject indicates absence of detectable SARS-CoV-2 IgG and IgM antibodies. However, a nonreactive test result does not preclude the possibility of exposure to or infection with SARS-CoV-2.
7. The risk of an asymptomatic person with reactive serum or plasma developing SARS-CoV-2 or, a SARS-CoV-2-related condition, is not known as the course of SARS-CoV-2 infections may vary among individual patients and may be altered by antiviral therapy.

- **INQUIRIES AND GENERAL INFORMATION**

  Please visit website www.dabimmunotech.com or
  email to info@dabimmunotech.com.

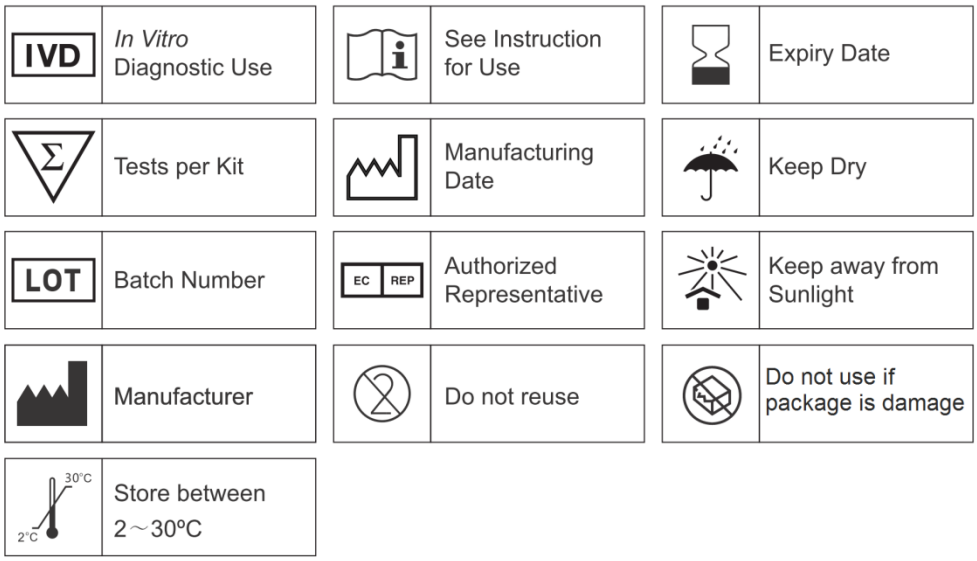
- **ORDERING**

  - Directly go to our website, www. dabimmunotech.com for online fast order.
  - Contact dAb ImmunoTech's distributors (See detail in our website).
  - Contact dAb ImmunoTech via email: order@dabimmunotech.com.

- **TECHNICAL**

  - Please reach us for technical questions and concerns via email, technical@dabimmunotech.com or
  - by phone, 470-566-1748(USA)

- **INDEX OF CE SYMBOLS**



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