SeroFlash™ SARS-CoV-2 IgM/IgG Antibody Detection Kit

Instructions for Use

Catalog # D-1001-025

25 Tests

Store between 2°C and 30°C

ALWAYS USE THE LATEST MANUAL INCLUDED WITH THE PRODUCT
FOR USE BY HEALTHCARE PROFESSIONALS OR RESEARCH SCIENTISTS ONLY

CE This product is manufactured under ISO 9001:2015 and ISO 13485:2016 standards, and meets the provisions of the EC Council Directives (In Vitro Diagnostic Medical Devices DIRECTIVE 98/79/EC).

EpiGentek FDA Owner/Operator Number: 10068270

Contents

A. Intended Use
B. Background and Principles of the Test
C. Kit Components
D. Materials Required but not Provided
E. Shipping and Storage Instructions
F. Sample Requirements
G. Quality Control
H. Detection Procedure
I. Detection Result Interpretation
J. Product Performance
K. Limitations and Precautions
L. Technical Assistance

A. Intended Use

The SeroFlash™ SARS-CoV-2 IgM/IgG Antibody Detection Kit is an immunoassay intended for the qualitative detection and differentiation of IgM and IgG antibodies to SARS-CoV-2 in human serum or whole blood. The SeroFlash™ SARS-CoV-2 IgM/IgG Antibody Detection Kit should not be used to diagnose acute SARS-CoV-2 infection.

Results indicate the presence of SARS-CoV-2 antibodies have been detected. While IgM and IgG antibodies to SARS-CoV-2 are generally detectable in blood for several days after initial infection, the duration that such antibodies are present for post-infection is not well characterized. The virus may be detectable in individuals for several weeks following seroconversion.

Laboratories within the United States and its territories should report positive results to appropriate public health authorities.

Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for the SeroFlash™ SARS-CoV-2 IgM/IgG Antibody Detection Kit may occur due to cross-reactivity from pre-existing antibodies or other possible causes. Due to the risks of false positive results, confirmation of positive results should be considered using an additional, different IgG or IgM assay.

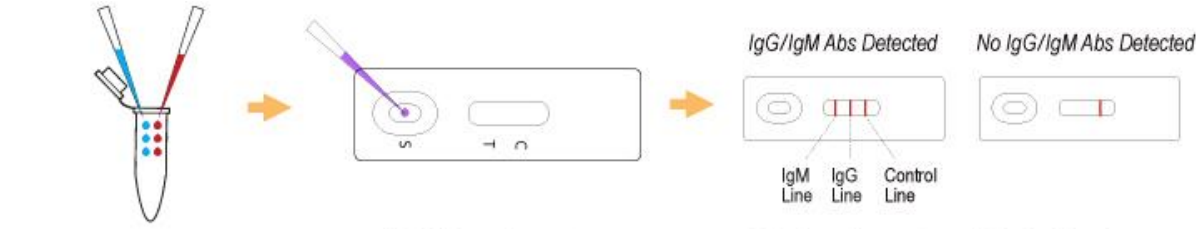
The SeroFlash™ SARS-CoV-2 IgM/IgG Antibody Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

B. Background and Principles of the Test

SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is a novel betacoronavirus and the cause of the COVID-19 disease. The genome of SARS-CoV-2 is a positive sense single-stranded ribonucleic acid (ssRNA) with a length of approximately 29.7 kb. SARS-CoV-2 is a new strain that has not been previously identified in humans. Individuals with SARS-CoV-2 report a mild to severe respiratory illness with symptoms of fever, cough, shortness of breath.

In the process of pathogenic microorganism infection, IgG and IgM are the most commonly used antibody markers of infectious diseases. IgM, as the first antibody in the process of infection, is usually used as a marker of acute infection. With the development of infection, IgM concentration gradually decreases and disappears after the appearance of IgG. IgG usually exists in the body for a long time, even if the virus has been completely eliminated. Thus, the presence of IgM and/or IgG antibodies to SARS-CoV-2 is a potential indicator of an immune response to infection. However, individuals can remain infectious in the presence of IgM and/or IgG if specimens are obtained during acute infection.

This kit uses lateral flow colloidal gold-based immunoassay technology by spraying colloidal gold-labeled recombinant SARS-CoV-2 antigen and a control antibody gold marker on a binding pad. Two test lines, G and M, and a control line C on the membrane are coated with mouse anti-human IgG (G), mouse anti-human IgM (M), and a quality control antibody (C), respectively. The M line is used to test for SARS-CoV-2 IgM antibody, the G line is used to test for SARS-CoV-2 IgG antibody, and the C line is used to check if the sample has been added to the sample well of the test card. The sample will subsequently move along the test card under the action of chromatography. If the sample contains the SARS-CoV-2 IgG or IgM antibody, the antibody binds to the gold labeled virus antigen. The immune complex forms a sandwich complex with the coated anti-human IgG or IgM monoclonal antibody at the G or M line, showing a purplish red G line or M line, respectively, and indicates a positive IgG or IgM antibody for SARS-CoV-2. If the test line G and M do not produce color, then a negative result is displayed. The pinkish red control line C on the card should appear regardless of whether there is a test line. If control line C does not appear, it indicates that the test result is invalid, and the sample should be tested again.



Combine serum or whole blood with Dilution Buffer in a tube and mix well.

Add diluted sample to the sample well of the test cartridge.

Monitor the test card for 3-15 minutes, then read results. Results that develop after 18 minutes are invalid.

C. Kit Components

Component	Size/Quantity	Composition
Test Card	25	PVC plastic vessel enclosing (a) a paper strip with membrane on which the detection area is coated with mouse anti-human IgG and mouse anti-human IgM antibodies and the control area is coated with a rabbit anti-chicken IgY antibody; (b) a combination pad sprayed with colloidal gold-labeled recombinant novel coronavirus antigen and chicken IgY antibody; (c) a sample pad; and (d) an absorbent pad
Sample Dilution Buffer	80 µl x 25 tubes*	Sample diluent, liquid phosphate buffer (pH 6.5-8.0)
Transfer Pipette	25*	Clear low-density polyethylene, sterilized
IFU Leaflet	1	Printed recycled paper

**** Note:** In actuality, one extra **Transfer Pipette** and **Simple Dilution Buffer** are included (26 totals of each) in case of spillage or user errors. Do not mix components from different kits or different lots.*

D. Materials Required but not Provided

- Blood collection tools and accessories
- Timer

E. Shipping and Storage Instructions

The kit is shipped at ambient room temperature. The **Test Card**, **Sample Dilution Buffer**, and **Transfer Pipette** can be stored between 2°C and 30°C after receipt (refrigerated or room temperature). The kit is stable for 12 months from the date of shipment, when stored properly. Refer to the kit labeling for expiry date. Test strips should be used within 20 minutes once the foil pouch is opened. See the label for the production date and service/shelf-life.

F. Sample Requirements

This assay is suitable for human serum and whole blood.

1. *Whole Blood Collection:* Drops of whole blood by venipuncture or fingertip puncture can be collected following laboratory procedure. The venipunctured whole blood should be collected into an EDTA, heparin, or sodium citrate anticoagulant tube. Immediately after specimen collection, shake up and down 5-10 times. Do not shake with force. The venipunctured whole blood samples can be stored at 2-8°C for 48 hours if it cannot be tested on a timely basis. Fingertip whole blood should be directly collected into the pre-aliquoted tube containing **Sample Dilution Buffer**, rather than a separate collection tube, and immediately used.

2. *Serum Collection:* After blood collection into serum separator collection tubes (without anticoagulant), serum should be immediately separated according to laboratory procedure to avoid hemolysis. If serum sample cannot be used on a timely basis, it can be stored at 2-8°C for 48 hours and at -20°C for 3 months. Fresh serum samples can be directly used for the test and frozen serum samples should be thawed at room temperature before use. Samples with severe lipid, hemolytic, or microbial contamination should not be used with this product. Turbidity of samples will affect the determination of results.

G. Quality Control

Internal Control: This test contains a built-in control, the C Line. The C Line develops after addition of the specimen and sample dilution buffer. If the C Line does not develop, the test is invalid. Review the procedure and repeat the test with a new card.

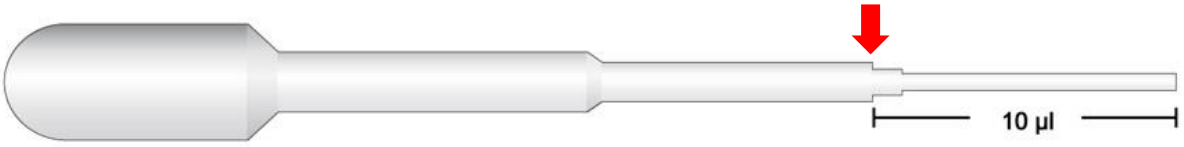
H. Detection Procedure

1. Restore the **Test Card** and **Sample Dilution Buffer** as well as your samples to room temperature (15-30°C) before testing.

2. Tear open the aluminum foil bag of the **Test Card**, take out the **Test Card** and place it horizontally on a flat lab surface.

3. Label or mark the test card with your test subject's identifier.

4. For serum and whole blood samples, carefully use the **Transfer Pipette** to pipette approximately 10 µl of sample (around the entire first segment of the fine tip of the pipette as shown) into the PCR tube containing **Sample Dilution Buffer**. For better precision the sample can be transferred by a laboratory micropipette capable of delivering 10 µl of volume.



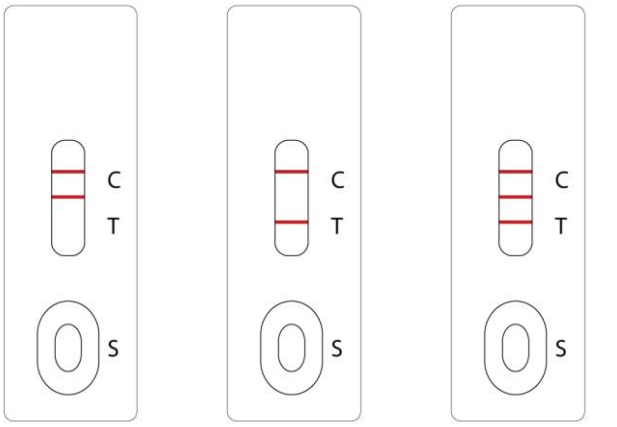
5. Centrifuge or tap down the contents on a hard surface. Mix by pipetting several times and then using the same **Transfer Pipette** transfer the entire 90 µl of the diluted sample into the sample well by slowly ejecting the mixture.

6. Read the results within 15 minutes. Any results read after 18 min are invalid.

I. Detection Result Interpretation

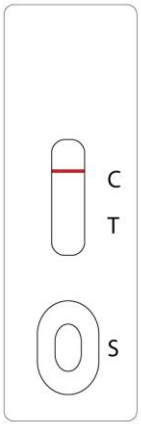
The **Test Card** is marked with C for control area and T for test area. For the test area, the 1st line from the sample well is IgM, followed by the IgG line between the C and T markings.

Positive Results: Both the G Line (between marker C and T on the test card) and the C Line (slightly above marker C on the test card) show color bands, indicating the sample is positive for SARS-CoV-2 IgG antibodies. Both the M Line (corresponding to marker T on the test card) and the C Line show color bands, indicating the sample is positive for SARS-CoV-2 IgM antibodies. Both M and G Lines as well as C Line all show color bands, indicating the sample is positive for both SARS-CoV-2 IgM and IgG antibodies.

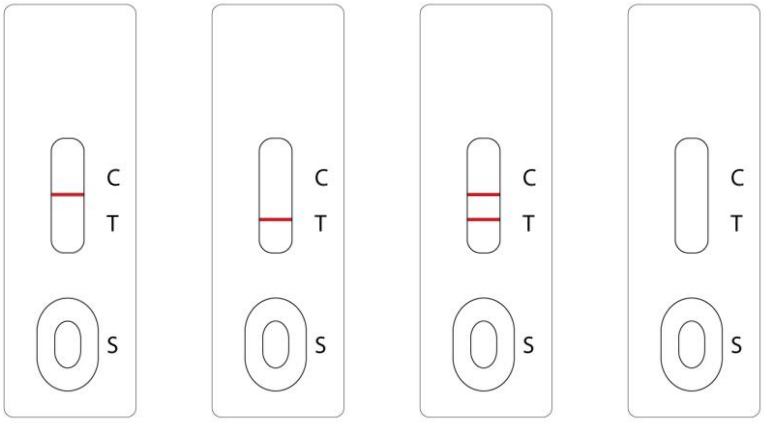


IgG Positive IgM Positive IgG & IgM Positive

Negative Results: If only the C Line shows color, but the G and M Lines do not, no IgM/IgG antibodies against the SARS-CoV-2 virus are detected, and the result is negative.



Invalid Results: No color band appears on the C Line. An invalid result is determined regardless of whether the G or M Lines show a band.



J. Product Performance

- *Negative Reference Product Compliance Rate:* 10 negative reference samples are tested for each Lot. The negative reference product compliance rate of the tested samples is 10/10.

- *Positive Reference Product Compliance Rate:* 10 positive reference samples are tested for each Lot. The positive reference product compliance rate of the tested samples is 10/10.

- *Minimum Test Limit:* 3 samples at differently diluted concentrations of antibodies are tested by adding 10 µl of sample to the sample well of the **Test Card**. Reference product S1 with the lowest concentration (very diluted: 0.5 ng/µl) is known negative and the assay confirmed S1 as negative. The reference products S2 (correct dilution: 12.5 ng/µl) and S3 (lower dilution: 3 ng/µl) are known positive and the assay confirmed S2 and S3 as positive.

- *Repeatability:* 10 negative and 10 positive reference products are tested from 2 different Lots of the kit. All negative products show negative and all positive products show positive.

- *Analysis Specificity:* The test was evaluated for potential cross-reactivity using serum or plasma samples containing antibodies to the pathogens listed below and the test had no cross-reaction to the listed pathogens:

  Local human coronaviruses (HKU1, OC43, NL63, and 229E)
  H1N1 (the new influenza A H1N1 virus-2019, seasonal H1N1); H3N2; H5N1; H7N9
  Influenza B/Yamagata and B/Victoria lineages
  Respiratory syncytial virus
  Rhinovirus A, B, and C groups
  Adenovirus types 1, 2, 3, 4, 5, 7, and 55
  Enterovirus A, B, C, D, and E groups
  EB virus
  Measles virus
  Human cytomegalovirus
  Rotavirus
  Norovirus
  Mumps virus
  Varicella zoster virus
  Mycoplasma pneumoniae

- *Potentially Endogenous Interfering Substances:* The following substances were spiked in serum/plasma samples known to contain antibodies to SARS-CoV-2 and had no interference to the test if the substances are less than the indicated concentrations.

Bilirubin	≤ 0.2 g/L
Hemoglobin	≤ 5 g/L
Triglycerides	≤ 10 g/L
Zanamivir	≤ 18 mg/L
Ribavirin	≤ 1.8 mg/L
Oseltamivir arboxylate	≤ 2.5 mg/L
Ceftriaxone	≤ 50 mg/L
Meropenem	≤ 3 mg/L
Arbidol	≤ 1 mg/L
Human IgG	≤ 7 g/L
Human IgM	≤ 0.4 g/L

- *Clinical Performance:* A preliminary study with clinical samples comprising 128 of whole blood samples from 103 of confirmed COVID-19 human subjects and 25 of healthy subjects showed the following correlation results:

Type	**# of Cases**	**Infection Time**	**Integrated Test**				**Correlation**		
			IgG	IgM	Any of IgG or IgM	Ctrl	IgG	IgM	Any of IgG or IgM
Positive	45	11-24 Days	42	44	44	45	93.33%	97.78%	**97.78%**
Positive	58	4-10 Days	19	49	49	58	32.76%	84.48%	**84.48%**
Negative	25	N/A	0	0	0	25	100%	100%	**100%**
Sensitivity: 90.29%; Specificity: 100%; Total Assay Accuracy: 92.19%									

Thus, in blood samples, the integrated IgG/IgM test has a 97.78% sensitivity for subjects with an infection window of 11-24 days, and an 84.48% sensitivity rate for subjects with an infection window of 4-10 days. The total assay accuracy for an infection window of 4-24 days is 92.19%.

In another preliminary study with clinical samples comprising 225 of serum samples from 130 of confirmed COVID-19 human subjects and 95 of healthy subjects showed the following correlation results:

Type	# of Cases	Infection Time	Integrated Test				Correlation		
			IgG	IgM	Any of IgG or IgM	Control	IgG	IgM	Any of IgG or IgM
Positive	69	11-24 Days	58	32	60	69	84.06%	46.38%	**86.96%**
Positive	61	4-10 Days	40	49	53	61	65.57%	78.69%	**86.89%**
Negative	95	N/A	1	0	1	95	98.9%	100%	**98.9%**
Sensitivity: 86.92%; Specificity: 98.9%; Total Assay Accuracy: 92.00%									

Thus, in serum samples, the integrated IgG/IgM test has an 86.96% sensitivity for subjects with an infection window of 11-24 days and an 86.89% sensitivity for subjects with an infection window of 4-10 days. The total assay accuracy for an infection window of 4-24 days is 92.00%.

K. Limitations and Precautions

- This kit is for *in vitro* diagnostic use only, following guidance from the FDA for Emergency Use Authorizations of such tests where applicable. This assay has not been reviewed by the FDA, and results from this test should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
- This kit is intended only for detection in human serum and whole blood samples.
- Any erroneous test results may be due to technical reasons, operational or performance errors, and other sample factors.
- Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals. During the early stage of infection, if the virus-specific IgM antibody is not produced or the titer is very low, it will lead to negative results. If virus infection is still suspected, the recipient should be prompted to be re-tested within 7-14 days.
- The test results of this product are only for clinical reference and should not be the sole basis for clinical diagnosis and treatment. The clinical management of recipients should be considered in combination with their symptoms/signs, medical history, other applicable laboratory tests, treatment response, epidemiology, and any other relevant information.
- Recipients with impaired immune function or receiving immunosuppressive therapy, such as those infected with human immunodeficiency virus (HIV) or receiving immunosuppressive therapy after organ transplantation, have limited reference value for serological IgM antibody detection, which may lead to incorrect medical interpretation.

- Positive test results should be carefully analyzed in persons who have received blood transfusions or other blood products in recent months.
- To prevent the possibility of virus infection during sample collection, wear disposable gloves, masks, and other PPE, as well as thoroughly wash your hands afterwards.
- The test shall be operated in strict accordance with these instructions and lab safety protocols.
- Do not use highly hemolytic and lipidic blood samples.
- Not for use of screening donated blood.
- Do not use this test beyond the expiration date.
- Damaged components or components with damaged packaging should not be used.
- This product does not provide a test report beyond the color lines generated by the **Test Cards**. When providing test report results to the recipient or appropriate party, you must advise the recipient of the following:
    - This test has not been reviewed by the FDA
    - Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals
    - Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status
    - Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E

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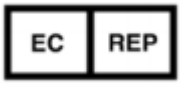
	Consult Instructions for Use		Manufacturer
	Lot Number		Use By
	For In Vitro Diagnostic Use Only		Tests Per Kit
	Catalog Number		Temperature Storage
	European Authorized Representative		Do not reuse
	CE Mark		