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COVID-19 IgG ELISA TEST SYSTEM

LOT EL1-1360 Σ 96 TESTS IVD

INTENDED USE

The Monocent, Inc.'s COVID-19 IgG ELISA Test System is intended for the detection of IgG antibodies to SARS-CoV-2 (COVID-19) in human serum or plasma.

SUMMARY AND EXPLANATION

Since late December 2019, an outbreak of a novel coronavirus disease (COVID-19; previously known as 2019-nCoV)1, was first reported in Wuhan, China,2 which has subsequently spread globally in a short period of time. COVID-19 or SARS-CoV-2 has demonstrated the capability to spread rapidly, leading to significant impacts on healthcare systems and causing societal disruption and the potential public health threat posed by COVID-19 is high3. In general, COVID-19 is an acute resolved disease, but it can also be deadly, with a 2% case fatality rate. COVID-19 is a single-stranded RNA coronavirus4. The viral infection causes a series of respiratory illness including severe respiratory syndrome, indicating the virus most likely infects respiratory epithelial cells and spreads mainly via respiratory tract from human to human4. Coronaviruses are composed of several proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N)5. Virus infections are characterized by elevations in specific IgM antibody levels 3 to 5 days after the onset of symptoms; this generally persists for 30 to 60 days. IgG levels also become elevated after 10 to 14 days and remain detectable for life.

PRINCIPLE OF THE TEST

Diluted patient serum is added to wells coated with purified COVID-19 antigen. SARS-CoV-2 IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away, and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the oxidation of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

MATERIALS AND COMPONENTS

1. Microwells coated with COVID-19 antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml
3. Calibrator: yellow Cap. 1 Vial (ready to use)	1ml
4. Positive Control: Red Cap. 1 vial (ready to use)	1ml
5. Negative Control: Blue Cap. 1 vial (ready to use)	1ml
6. Enzyme conjugate: 1 bottle (ready to use)	13ml
7. TMB Substrate: 1 bottle (ready to use)	13ml
8. Stop Solution: 1 bottle (ready to use)	13ml
9. Wash concentrate 20X: 2 bottles	2x25ml

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision pipettes
2. Disposable pipette tips
3. ELISA reader capable of reading absorbance at 450nm
4. Flat-head Vortex mixer
5. Plate shaker
6. Graph paper

STORAGE CONDITIONS

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
2. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas where specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8°C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.
3. **Warning:** Do not use IgM Sample Diluent for IgG testing.

REAGENT PREPARATION

Before running the test, prepare the following:
Wash Buffer: Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (Average of 23°C).

TEST PROCEDURE

All reagents and specimens must be allowed to come to room temperature before use and must be GENTLY mixed without foaming. Once the procedure has started, all steps should be completed without interruption.

1. Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.
2. Place the desired number of coated strips into the holder.
3. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
4. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
5. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µL of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 10 min. A dual wavelength is recommended with reference filter of 600-650 nm.

CALCULATION OF RESULTS

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the mean values of each sample by cut-off value.

EXAMPLE OF TYPICAL RESULTS

Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5 = 0.400
Positive Control OD= 1.2
Ab Index = 1.2 / 0.4 = 3
Patient Sample OD = 1.6
Ab Index = 1.6 / 0.4 = 41.0

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The OD of the Calibrator should be greater than 0.250
2. The Ab Index for Negative control should be less than 0.9
3. The Ab Index for Positive control should fall within the range specified on the COA/Label.

INTERPRETATION

The following is intended as a guide to interpretation of COVID-19 IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

ANTIBODY INDEX INTERPRETATION

< 0.9	No detectable IgG Antibody to COVID-19.
0.9 - 1.1	Borderline Positive. Follow-up testing is recommended if clinically indicated.
> 1.1	Detectable IgG antibody to COVID-19.

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.
3. This test Is only provided for use by clinical and not for at home testing.
4. This test has not been reviewed by the FDA.
5. 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.
6. 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.
7. 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.
8. Not for the screening of donated blood.

PERFORMANCE CHARACTERISTICS

1. Sensitivity & Specificity

Serum samples collected from previously RT-PCR confirmed COVID-19 patients were tested in Monocent's ELISA Test System. 40 Normal healthy patients with samples collected before COVID-19 outbreak (prior to December 2019) were tested in Monocent's ELISA. The results are as follows:

	Test Positive	Test Negative
Confirmed Positive	17	0
Confirmed Negative	0	40

The Sensitivity is 100%
The Specificity is 100%

2. Class Specificity

This assay does not show any cross reaction to IgM.

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COVID-19 IgM NOVEL CORONAVIRUS ELISA TEST SYSTEM

REF EL36-1361R **96 TESTS**

INTENDED USE

The Monocent, Inc.'s COVID-19 IgM ELISA Test System is intended for the qualitative detection of human anti-COVID19 IgM antibody in human serum.
This ELISA Test System is used as an aid for the detection of novel COVID-19. Patients with suspected clustering cases require diagnosis or differential diagnosis of novel coronavirus infection.

SUMMARY AND EXPLANATION

2019 novel coronavirus (COVID-19) is a single-stranded RNA coronavirus2. Comparisons of the genetic sequences of this virus have shown similarities to SARS-CoV and bat coronaviruses7. In humans, coronaviruses cause respiratory infections3. Coronaviruses are composed of several proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N)4. Results suggest that the spike protein retains sufficient affinity to the Angiotensin converting enzyme 2 (ACE2) receptor to use it as a mechanism of cell entry6. Human to human transmission of coronaviruses is primarily thought to occur among close contacts via respiratory droplets generated by sneezing and coughing1. IgM is the first immunoglobulin to be produced in response to an antigen and will be primarily detectable during the early onset of the disease5.

PRINCIPLE OF THE TEST

Monocent, Inc.'s ELISA Test System is designed, developed, and produced for the qualitative measurement of the COVID-19 IgM antibody in serum. This assay utilizes the "IgM capture" method on microplate based enzyme immunoassay technique.
Assay controls and samples are added to the microtiter wells of a microplate that was coated with a anti-human IgM specific antibody. After the first incubation period, the unbound protein matrix is removed with a subsequent washing step. A horseradish peroxidase (HRP) labeled recombinant COVID-19 antigen is added to each well. After an incubation period, an immunocomplex of "Anti-hIgM antibody - human COVID-19 IgM antibody - HRP labeled COVID-19 antigen" is formed if there is novel coronavirus IgM antibody present in the tested materials. The unbound tracer antigen is removed by the subsequent washing step. HRP-labeled COVID-19 antigen tracer bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antigen bound to the coronavirus IgM on the wall of the microtiter well is proportional to the amount of the coronavirus IgM antibody level in the tested materials.

MATERIALS AND COMPONENTS

1. COVID-19 IgM Microplate
Microplate coated with anti-human IgM specific antibody.
Qty: 1 x 96 well microplate, Ready to use.
2. COVID-19 IgM Sample Diluent
A ready-to-use sample dilution buffer.
Qty: 1 x 15 mL.
3. HRP Labeled COVID-19 Antigen
HRP labeled COVID-19 Antigen in a stabilized protein matrix. Qty: 1 x 11 mL, Ready to use.
4. ELISA Wash Concentrate
Surfactant in a phosphate buffered saline with non-azide preservative.
Qty: 1 x 30 mL, 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.
5. ELISA HRP Substrate
Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Qty: 1 x 15 mL, Ready to use.
6. ELISA Stop Solution
0.5 M sulfuric acid.
Qty: 1 x 15 mL, Ready to use.
7. COVID-19 IgM Negative Control
Negative control with a bovine serum albumin based matrix with non-azide preservative. Control products do not contain any serum from patients with new type of coronavirus infection. Qty: 1 x 1 mL, Ready to use.
8. COVID-19 IgM Positive Control
Positive control with a bovine serum albumin based matrix with non-azide preservative. Control products do not contain any serum from patients with new type of coronavirus infection. Qty: 1 x 0.5 mL, Ready to use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 20 µL, 25 µL, 100 µL, and 1000 µL, etc.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm or 13 x 100 glass tubes.
5. Disposable plastic 1000 mL bottle with caps.
6. Aluminum foil.
7. Deionized or distilled water.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.
11. Incubator capable of holding the temperature at 37 ºC.

STORAGE CONDITIONS

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

PRECAUTIONS

Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

SPECIMEN COLLECTION

Only 20 µL of human serum is required for measurement in duplicate. Samples should only be used on the same day. Severe hemolytic samples should not be used.

REAGENT PREPARATION

Reagent Preparation
1. Prior to use, allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate must be diluted to working solution prior to use. Please see MATERIALS AND COMPONENTS section for details.

TEST PROCEDURE

1. Place a sufficient number of microwell strips in a holder to run controls and samples in duplicate.
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Negative Control	SAMPLE 3	SAMPLE 7
B	Negative Control	SAMPLE 3	SAMPLE 7
C	Negative Control	SAMPLE 4	SAMPLE 8
D	Positive Control	SAMPLE 4	SAMPLE 8
E	SAMPLE 1	SAMPLE 5	SAMPLE 9
F	SAMPLE 1	SAMPLE 5	SAMPLE 9
G	SAMPLE 2	SAMPLE 6	SAMPLE 10
H	SAMPLE 2	SAMPLE 6	SAMPLE 10

3. Add 100 µL of controls into the designated microwells.
4. Add 10 µL of samples into the designated microwells.
5. Add 100 µL of COVID-19 IgM Sample Diluent to the microwells with the samples. Note: Do not add sample diluent to the wells

with the controls!

6. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at Incubate at 37 ℃ for 30 minutes.
7. Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
8. Add 100 µL of the HRP-labeled COVID-19 antigen into the microwells.
9. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at Incubate at 37 ℃ for 30 minutes.
10. Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of diluted wash solution into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
11. Add 100 µL of the substrate into the microwells.
12. Mix gently and cover the plate with aluminum foil. Incubate at room temperature (20-25 ℃) for 20 minutes.
13. Remove the aluminum foil and add 100 µL of stop solution into each of the microwells. Mix by gently tapping the plate.
14. Read the absorbance at 450 nm within 10 minutes with a microplate reader.

PROCEDURAL NOTES

1. It is recommended that all samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original bottles and avoid unnecessary exposure to the light.
3. Store any unused antibody-coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

QUALITY CONTROL

To assure the validity of the results each assay must include both negative and positive controls. The average of the negative control absorbance values less than 0.25 and the positive control absorbance value is not less than 0.50.We also recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

INTERPRETION OF RESULTS

1. Calculate the average value of the absorbance of the negative control (xNC).
2. Calculate the Background Adjustment Factor (BAF) using the following formulas:
   - Positive cutoff = 1.1 X (xNC + 0.10)
   - Negative cutoff = 0.9 x (xNC + 0.10)
3. Determine the interpretation of the sample by comparing the OD to the following table:

Interpretation	Interval	Results
Negative	Measured value ≤ negative cutoff	The sample does not contain the new coronavirus (COVID-19) IgM related antibody.
Positive	Measured value ≥ positive cutoff	The sample contains novel coronavirus (COVID-19) IgM associated antibodies.
Borderline	Negative cutoff < Measured value < Positive cutoff	Retest the sample in conjunction with other clinical tests.

PERFORMANCE CHARACTERISTICS

Limit of Detection

The limit of detection is not higher than 5 U/mL.

Repeatability

The assay control is tested in 10 replicates with a CV of OD values less than 15%.

Reproducibility

Three lots were tested with the same samples 10 times with a CV less than 20%.

LIMITATIONS OF THE TEST

1. This test is only for qualitative detection. Test results should not be the sole basis for clinical diagnosis and treatment. The confirmation of infection with novel coronavirus (COVID-19) must be combined with the patient's clinical signs in conjunction to other tests.
2. In the first week of the onset or after four weeks of the infection novel coronavirus (COVID-19) patients may be negative for IgM. In addition, patients with low immunity or other diseases that affect immune function, failure of important systemic organs, and use of drugs that suppress immune function can also lead to negative results of new coronavirus IgM.
3. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
4. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.
5. This test has not been reviewed by the FDA.
6. 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.
7. 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.
8. Positive results may be due to past or present infection with nonSARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
9. Not for the screening of donated blood.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Monocent Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Monocent Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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