EPITOPE DIAGNOSTICS, INC.

EDI™ Novel Coronavirus COVID-19 IgM ELISA Kit

Enzyme Linked Immunosorbent Assay (ELISA) for the qualitative detection of the COVID-19 IgM in human serum.

REF KTR-1033 RUO CANADA 96

INTENDED USE

This kit is for research use only. The kit is detecting novel COVID-19 IgM antibody in human serum. It is for screening or to aid in the diagnosis of COVID-19. Patients with suspected clustering cases require diagnosis or differential diagnosis of novel coronavirus infection. The assay is validated manually, but can be adapted to an automated instrument. The assay is for the qualitative detection only.

SUMMARY OF PHYSIOLOGY

2019 novel coronavirus (COVID-19) is a single-stranded RNA coronavirus[2]. Comparisons of the genetic sequences of this virus have shown similarities to SARS-CoV and bat coronaviruses[7]. In humans, coronaviruses cause respiratory infections[3]. 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 entry[6]. Human to human transmission of coronaviruses is primarily thought to occur among close contacts via respiratory droplets generated by sneezing and coughing[1]. IgM is the first immunoglobulin to be produced in response to an antigen and will be primarily detectable during the early onset of the disease[5].

ASSAY PRINCIPLE

This ELISA kit 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.

REAGENTS: PREPARATION AND STORAGE

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.

1. COVID-19 IgM Microplate (31223)

Microplate coated with anti-human IgM specific antibody.

Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to use.

2. COVID-19 IgM Sample Diluent (31224)

A ready-to-use sample dilution buffer.

Qty: 1 x 15 mL
Storage: 2 – 8°C
Preparation: Ready to use.

3. HRP Labeled COVID-19 Antigen (31226)

HRP labeled COVID-19 Antigen in a stabilized protein matrix.

Qty: 1 x 11 mL
Storage: 2 – 8°C
Preparation: Ready to use.

4. ELISA Wash Concentrate (10010)

Surfactant in a phosphate buffered saline with non-azide preservative.

Qty: 1 x 30 mL
Storage: 2 – 25°C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

5. ELISA HRP Substrate (10020)

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

Qty: 1 x 15 mL
Storage: 2 – 8°C
Preparation: Ready to use.

6. ELISA Stop Solution (10030)

0.5 M sulfuric acid.

Qty: 1 x 15 mL
Storage: 2 – 25°C
Preparation: Ready to use.

7. COVID-19 IgM Negative Control (31228)

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
Storage: 2 – 8°C.
Preparation: Ready to use.

8. COVID-19 IgM Positive Control (31229)

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
Storage: 2 – 8°C.
Preparation: Ready to use.

SAFETY PRECAUTIONS

The reagents are for research use only. 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.

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.

SAMPLE COLLECTION & STORAGE

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.

ASSAY PROCEDURE

1. 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 (10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.

2. Assay Procedure

1. Place a sufficient number of microwell strips (31223) in a holder to run controls (31228, 31229) 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	SAMPLE4	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 (31228, 31229) into the designated microwells.
4. Add **10 µL** of samples into the designated microwells.
5. Add **100 µL** of COVID-19 IgM Sample Diluent (31224) 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 ºC** 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 (10010) 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 (31226) into the microwells.
9. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at Incubate at **37 ºC** 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 (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
11. Add **100 µL** of the substrate (10020) into the microwells.
12. Mix gently and cover the plate with aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
13. Remove the aluminum foil and add **100 µL** of stop solution (10030) 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 cutoffs 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) an IgM - associated antibodies.
Borderline	Negative cutoff < Measured value < Positive cutoff	Retest the sample in conjunction with other clinical tests.

EXPECTED VALUES

Samples from the clinical testing presented ODs of 0.164 – 0.661 for the positive values and 0.000 – 0.151 for the negative values. These values should not be in lieu of the interpretation of results calculation.

LIMITATIONS OF THE PROCEDURE

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.

PERFORMANCE CHARACTERISTICS

Assay Development

This assay was developed by evaluating eight commercially available COVID-19 antigens to screen for optimal use in this serological test. The assays were first evaluated with normal healthy donor serum samples to obtain negative test results. The assays were further evaluated with 20 positive serum samples from confirmed COVID-19 patients tested by RT-PCR. The best performing antigen was selected for the development of the kit**.**

Limit of Detection

Three lots of material were tested with one assay using a blank control in sixteen replicates. LoD was calculated as the mean of the OD for the blank control plus three times the standard deviation. The highest of the three runs was established for the LoD at 0.0669. The results are as follows:

	Average OD (450 nm)	CV (%)	LOD (x? + 3 SD)
Run 1	0.0560	5.32%	0.0649
Run 2	0.0568	5.63%	0.0663
Run 3	0.0561	6.46%	0.0669

Repeatability

One lot of material was tested with one assay using three samples (strong positive, light positive, and negative) in sixteen replicates. For all sixteen replicates, sample 1 and 2 are positive and in sample 3 is all negative. The repeatability results are very satisfactory with acceptable CV. The results are as follows:

ID	Average OD (450 nm)	Results	CV (%)
Sample 1	1.023	16/16 are Positive	4.48%
Sample 2	0.443	16/16 are Positive	4.83%
Sample 3	0.125	16/16 are Negative	9.17%

Reproducibility

One lot of material was tested over twelve assays using three samples (strong positive, light positive, and negative) in two replicates and a set of positive and negative controls in three replicates. For all twelve assays, sample 1 and 2 are positive and sample 3 is all negative. The results for reproducibility are very satisfactory with an acceptable CV. The results are as follows:

ID	Average OD (450 nm)	Results	CV (%)
Sample 1	1.18	12/12 are Positive	1.93%
Sample 2	0.53	12/12 are Positive	2.37%
Sample 3	0.13	12/12 are Negative	3.32%
Negative Control	0.09	12/12 are Negative	3.92%
Positive Control	0.89	12/12 are Positive	3.49%

Class Specificity

Five RT-PCR confirmed samples with positive COVID-19 IgG were tested in duplicate in both IgM and IgG kit. The results showed that there is no cross reaction to IgG in this COVID-19 IgM test. This assay is specific to the detection of IgM even in samples of positive COVID-19 IgG. The results are as follows:

Sample ID	IgM Result	IgG Result
Sample 1	-	+
Sample 2	-	+
Sample 3	-	+
Sample 4	-	+
Sample 5	-	+

Cross-Reactivity

Panels were studied with confirmed disease state samples. The selection was based on recommendations from the FDA EUA Program. Per the recommendation, Anti-haemophilus influenzae and Anti-Rhinovirus were recommended but unable to be tested. Additionally, a large number of panel samples are tested in a Normal US population Study from a population with a high prevalence of vaccination against influenza, HBV, and Haemophilus influenzae, and specificity of 100% was observed. No interference was observed for the following disease or infectious agents:

Agent	Disease State Confirmation Test
Influenza A	Viron/Serion
Influenza B	Viron/Serion
Respiratory syncytial virus	EIA
Hepatitis C Virus	Roche Ampliprep/Taqman
Antinuclear Antibodies	Bio-Rad Hep 2
Human Immunodeficiency Virus	Innogenetics
Hepatitis B Virus	Siemens

Normal US Population

Serum samples were tested at a laboratory in the United States. The samples consisted of 100 males and females from ages 19 - 65. These samples were collected for testing after the outbreak of COVID-19 and were not screened using additional testing methods. Very satisfactory results were obtained with 100% clinical diagnostic specificity. The results are as follows:

	Number of Samples
IgM Positive	0
IgM Negative	100
IgM Equivocal	0

Specificity	100%

Transportation Stability

One lot of material was shipped from Epitope Diagnostics, Inc. in San Diego, CA to an external site in the United States and returned. The kit was packaged in a foam box with blue ice which was not changed for the duration of the study to simulate transport conditions. The kits were in this condition for a total of 31 days. A comparison of the values obtained before and after shipment demonstrates the stability of the materials. The results are as follows:

Before Shipment			After Shipment		
Well ID	OD	Average	Well ID	OD	Average
Negative	0.098	0.10333333	Negative	0.091	0.09333333
	0.101			0.089	
	0.111			0.091	
Positive	1.387	N/A	Positive	0.868	N/A
Neg. Cut-off	0.255		Neg. Cut-off	0.243	
Pos. Cut-off	0.312		Pos. Cut-off	0.297	

CLINICAL TESTING

Serum Samples were tested using the IgM ELISA kit at the Jiaxing City Center for Disease Control and Prevention and a laboratory in the United States. The combined cohort consisted of normal healthy patients with samples collected prior to the COVID-19 outbreak [December 3, 2019] (n=54) and RT-PCR confirmed positive patients (N = 40). Lower rates of sensitivity can be attributed to the date of collection versus the onset of symptoms. The results are as follows:

	PCR Test Positive	PCR Test Negative
IgM Test Positive	30	0
IgM Test Negative	8	54
IgM Test Borderline	2	0

IgM is the first immunoglobulin to be produced in response to an antigen and will be primarily detectable during the early onset of the disease. The National Health Commission of the People's Republic of China states that IgM antibodies begin to show positive after 3-5 days of onset of COVID-19. Serum samples for the clinical test were from patients after two weeks of the onset of the disease. Therefore, low levels of clinical sensitivity for IgM can be attributed to the collection date of the positive cohort where IgM levels are expected to be lower.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, 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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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

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Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)