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 serum.

REF KT-1033 IVD CE 96 +2 +8 °C

INTENDED USE

This kit is for in vitro diagnostics use only. The kit is detecting novel COVID-19 Nucleocapsid protein specific 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.

INTENDED USER

This kit is for laboratory professional use or healthcare professionals.

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 (ACE-2) 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 12 mL
Storage: 2 – 8°C
Preparation: Ready to use

6. ELISA Stop Solution (10030)
0.5 M sulfuric acid.
Qty: 1 x 12 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 in-vitro diagnostic 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 10 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 plastic 1000 mL bottle with caps.
5. Aluminum foil.
6. Deionized or distilled water.
7. Plastic microtiter well cover or polyethylene film.
8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
9. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.
10. 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 negative control (31228) in triplicate, positive control (31229) in singlet, and samples in duplicate.

   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

2. Add **100 µL** of controls (31228, 31229) into the designated microwells.
3. Add **10 µL** of samples into the designated microwells.
4. 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!
5. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at Incubate at **37 ºC** for **30 minutes.**
6. 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.
7. Add **100 µL** of the HRP-labeled COVID-19 antigen(31226) into the microwells.
8. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at Incubate at **37 ºC** for **30 minutes.**
9. 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.
10. Add **100 µL** of the substrate (10020) into the microwells.
11. Mix gently and cover the plate with aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
12. Remove the aluminum foil and add **100 µL** of stop solution (10030) into each of the microwells. Mix by gently tapping the plate.
13. 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 recommends 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 cut off = 1.1 x (xNC + 0.10)
   - Negative cut off = 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 cut-off	The sample doesn't contain COVID-19 IgM antibodies
Positive	Measured value ≥ positive cut-off	The sample contains COVID-19 IgM antibodies
Borderline	Negative cut-off < measured value < positive cut-off	Retest the sample in conjunction with other clinical tests

4. Race and geographical region may affect the results from normal donor samples. Laboratories may establish or modify the cutoff based upon additional validation.

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.

Reactivity/Inclusivity

Although mutations in the SARS-CoV-2 genome have been identified as the virus has spread, no serologically unique strains have been described relative to the originally isolated virus (this research is exceptionally limited at present).

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:

Run	Average OD at 450 nm	CV (%)	LOD ($\bar{x}$ + 3 SD)
1	0.0560	5.32%	0.0649
2	0.0568	5.63%	0.0663
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 results are shown below with acceptable CV.

Sample	Average OD at 450 nm	CV (%)	Results
1	1.023	4.48%	16/16 (+)
2	0.443	4.83%	16/16 (+)
3	0.125	9.17%	16/16 (-)

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 are shown below with an acceptable CV.

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

Cross-Reactivity

A large number of known negative samples (N=80) collected in the US prior to December 2019 were tested from a population with a high prevalence of vaccination against, and/or infection with, the following viruses, and specificity of 100% is observed, cross-reactivity testing for the following viruses would not be expected at this time:

Anti-influenza A (IgG and IgM)	Anti-229E (alpha coronavirus)
Anti-influenza B (IgG and IgM)	Anti-NL63 (alpha coronavirus)
Anti-HCV (IgG and IgM)	Anti-OC43 (beta coronavirus)
Anti-HBV (IgG and IgM)	Anti-HKU1 (beta coronavirus)
Anti-haemophilus influenzae (IgG and IgM)	Anti-respiratory syncytial virus (IgG and IgM)
Antinuclear Antibody	Anti-HIV

To demonstrate cross-reactivity of the test, Epitope Diagnostics, Inc. used the FDA required minimum of 5 individual samples tested in duplicate for each disease/infectious agent using natural specimen confirmed with commercially available diagnostic tests. All samples were sourced from natural specimens using sera from patients with the underlying diseases in the acute or convalescent stages of infection. The disease and Infection agents were selected based on recommendations from the FDA EUA Program. The recommendation also included Anti-haemophilus influenzae and rhinovirus, but this material was unable to be tested due to lack of availability.

Agent	Confirmation Test	Results
Influenza A	Viron/Serion	5/5, negative
Influenza B	Viron/Serion	5/5, negative
Respiratory Syncytial Virus	EIA. Viron/Serion	5/5, negative
Hepatitis B Virus	Siemens	5/5, negative
Hepatitis C Virus	Roche Ampliprep/Taqman	5/5, negative
Antinuclear Antibody	Bio-Rad Hep 2	5/5, negative

Class Specificity

To evaluate class specificity, Ten RT-PCR confirmed COVID-19 patient serum samples were tested in duplicate in the Epitope Diagnostics, Inc. IgG and IgM ELISA Kits. Samples 1 - 5 are IgM positive and IgG negative. Sample 1 is a natural and untreated IgM positive, IgG negative. Sample 2 - 5 were originally positive for IgG and IgM but used protein A/ProSep A to remove the IgG. Samples 6 -10 are IgG positive and IgM negative. All samples 6 - 10 are natural and untreated IgG positive, IgM negative. There is 100% agreement between the results of this test. This demonstrates that the assay is specific to the detection of IgM class without cross reaction to COVID-19 IgG class.

The results are as follows:

Sample ID	IgM Result	IgG Result	Sample ID	IgM Result	IgG Result
Sample 1	(+)	(-)	Sample 6	(-)	(+)
Sample 2	(+)	(-)	Sample 7	(-)	(+)
Sample 3	(+)	(-)	Sample 8	(-)	(+)
Sample 4	(+)	(-)	Sample 9	(-)	(+)
Sample 5	(+)	(-)	Sample 10	(-)	(+)

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	0.103	Negative	0.091	0.090
	0.101			0.089	
	0.111			0.091	
Positive	1.387	N/A	Positive	0.868	N/A
Negative Cut-off	0.255		Negative Cut-off	0.243	
Positive Cut-off	0.312		Positive Cut off	0.297	

CLINICAL TESTING

Patient samples were tested using the IgM ELISA kit at five sites: Center for Disease Control and Prevention in China, a University Hospital in China, and a laboratory in the United States and three Canadian laboratories. The combined cohort consisted of normal healthy patients with samples collected prior to the COVID-19 outbreak (N=233) and RT-PCR confirmed positive patients (N=130).

The results are as follows:

Days from symptoms onset	Number of samples tested	IgM positive results	IgM PPA	95% CL
0-7 days	24	13	54.2%	0.3507 – 0.7211
8-14 days	55	43*	78.2%	0.6563 – 0.8705
15-30 days	51	47	92.2%	0.8150 – 0.9691

**Equivalent results being interpreted as positive*

Days from symptoms onset	Number of samples tested	IgM positive results	IgM PPA	95% CL
0-7 days	24	13	54.2%	0.3507 – 0.7211
8-14 days	55	42**	76.4%	0.6365 – 0.8563
15-30 days	51	47	92.2%	0.8150 – 0.9691

***Equivalent results being interpreted as negative*

Days from symptoms onset	Number of samples tested	IgM positive results	IgM NPA	95% CL
Pre-pandemic samples	233	233	100%	0.976 – 1.000

Generally, 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 changing of PPA in different dates from symptom onset of COVID-19 may provide information of patient immune response.

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 +1 (858) 693-7877, fax to +1 (858) 693-7678 or email at cs@epitopediagnostics.com

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)