**INTENDED USE**
This kit is intended for the qualitative detection of human anti-COVID-19 IgM antibody in human serum.

**INDICATIONS FOR USE**
This kit is to be 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. This kit is being distributed under Section IV.D of Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency. This kit is only provided for use by clinical laboratories or to healthcare workers for point-of-care testing, and not for at home testing.

The kits are registered under product code QKO, our submission number is D376537. The establishment registration number is 2032839.

**SUMMARY OF PHYSIOLOGY**
2019 novel coronavirus (COVID-19) is a single-stranded RNA coronavirus. Comparisons of the genetic sequences of this virus have shown similarities to SARS-CoV and bat coronaviruses. In humans, coronaviruses cause respiratory infections. Coronaviruses are composed of several proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N). 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. Human to human transmission of coronaviruses is primarily thought to occur among close contacts via respiratory droplets generated by sneezing and coughing. 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.

**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 well 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.

**ASSAY PRINCIPLE**
This ELISA kit is designed, developed, and produced for the qualitative detection of the COVID-19 IgM in serum. This ELISA kit utilizes the “IgM capture” method on microplate based enzyme immunoassay technique.

**REAGENTS: PREPARATION AND STORAGE**
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**INTENDED USE**
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**SUMMARY OF PHYSIOLOGY**
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**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 well 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.

**ASSAY PRINCIPLE**
This ELISA kit is designed, developed, and produced for the qualitative detection of the COVID-19 IgM in serum. This ELISA kit utilizes the “IgM capture” method on microplate based enzyme immunoassay technique.

**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.
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 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 negative control (31228) in triplicate, positive control (31229) in singlet, and samples in duplicate.
   2. Test Configuration
      
<table>
<thead>
<tr>
<th>Row</th>
<th>Strip 1</th>
<th>Strip 2</th>
<th>Strip 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Negative Control</td>
<td>SAMPLE 3</td>
<td>SAMPLE 7</td>
</tr>
<tr>
<td>B</td>
<td>Negative Control</td>
<td>SAMPLE 3</td>
<td>SAMPLE 7</td>
</tr>
<tr>
<td>C</td>
<td>Negative Control</td>
<td>SAMPLE 4</td>
<td>SAMPLE 8</td>
</tr>
<tr>
<td>D</td>
<td>Positive Control</td>
<td>SAMPLE 4</td>
<td>SAMPLE 8</td>
</tr>
<tr>
<td>E</td>
<td>SAMPLE 1</td>
<td>SAMPLE 5</td>
<td>SAMPLE 9</td>
</tr>
<tr>
<td>F</td>
<td>SAMPLE 1</td>
<td>SAMPLE 5</td>
<td>SAMPLE 9</td>
</tr>
<tr>
<td>G</td>
<td>SAMPLE 2</td>
<td>SAMPLE 6</td>
<td>SAMPLE 10</td>
</tr>
<tr>
<td>H</td>
<td>SAMPLE 2</td>
<td>SAMPLE 6</td>
<td>SAMPLE 10</td>
</tr>
</tbody>
</table>

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 °C for 30 minutes.
7. Remove the plate sealer. Aspirate the contents of each well. Oxyge 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 °C 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 each microwell.
12. Mix gently and cover the plate with aluminum foil. Incubate at room temperature (20-25 °C) 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.

INTERPRETATION 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 × (xNC + 0.10)
   - Negative cutoff = 0.9 × (xNC + 0.10)
3. Determine the interpretation of the sample by comparing the OD to the following table:

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Interval</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Measured value ≤ negative cutoff</td>
<td>The sample does not contain the new coronavirus (COVID-19) IgM-related antibody</td>
</tr>
<tr>
<td>Positive</td>
<td>Measured value ≥ positive cutoff</td>
<td>The sample contains novel coronavirus (COVID-19) IgM-associated antibodies</td>
</tr>
<tr>
<td>Borderline</td>
<td>Negative cutoff &lt; Measured value &lt; Positive cutoff</td>
<td>Retest the sample in conjunction with other clinical tests</td>
</tr>
</tbody>
</table>

KT-1033/ IVD,US/ V2/2020-05
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.

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.

7. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.

8. 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.

9. 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.

**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:

<table>
<thead>
<tr>
<th>ID</th>
<th>Average OD (450 nm)</th>
<th>CV (%)</th>
<th>LOD (x7 + 3 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run 1</td>
<td>0.0560</td>
<td>5.32%</td>
<td>0.0649</td>
</tr>
<tr>
<td>Run 2</td>
<td>0.0568</td>
<td>5.63%</td>
<td>0.0663</td>
</tr>
<tr>
<td>Run 3</td>
<td>0.0561</td>
<td>6.49%</td>
<td>0.0669</td>
</tr>
</tbody>
</table>

**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:

<table>
<thead>
<tr>
<th>ID</th>
<th>Average OD (450 nm)</th>
<th>Results</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>1.023</td>
<td>16/16 are Positive</td>
<td>4.48%</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.443</td>
<td>16/16 are Positive</td>
<td>4.83%</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0.125</td>
<td>16/16 are Negative</td>
<td>9.17%</td>
</tr>
</tbody>
</table>

**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:

<table>
<thead>
<tr>
<th>ID</th>
<th>Average OD (450 nm)</th>
<th>Results</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>1.18</td>
<td>12/12 are Positive</td>
<td>1.93%</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.53</td>
<td>12/12 are Positive</td>
<td>2.37%</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0.13</td>
<td>12/12 are Negative</td>
<td>3.32%</td>
</tr>
<tr>
<td>Negative Control</td>
<td>0.09</td>
<td>12/12 are Negative</td>
<td>3.92%</td>
</tr>
<tr>
<td>Positive Control</td>
<td>0.89</td>
<td>12/12 are Positive</td>
<td>3.49%</td>
</tr>
</tbody>
</table>

**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:

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>IgM Result</th>
<th>IgG Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample 2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sample 3</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sample 4</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sample 5</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sample 6</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sample 7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample 8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample 9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample 10</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Cross-Reactivity**

A large number of known negative samples (N=624) 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 96.8% is observed, cross-reactivity testing for the following viruses would not be expected at this time:

- anti-influenza A (IgG and IgM)
- anti-influenza B (IgG and IgM)
- anti-HCV (IgG and IgM)
- anti-HBV (IgG and IgM)
- anti-Haemophilus influenzae (IgG and IgM)
- anti-229E (alpha coronavirus)
- anti-NL63 (alpha coronavirus)
- anti-OC43 (beta coronavirus)
- anti-HKU1 (beta coronavirus)
- ANA
- anti-respiratory syncytial virus (IgG and IgM)
- 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.
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:

<table>
<thead>
<tr>
<th></th>
<th>Before Shipment</th>
<th>After Shipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well ID</td>
<td>OD Average</td>
<td>Well ID</td>
</tr>
<tr>
<td>Negative</td>
<td>0.098</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>0.101</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>0.111</td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>1.387</td>
<td>Positive</td>
</tr>
<tr>
<td>Neg. Cut-off</td>
<td>0.255</td>
<td>Neg. Cut-off</td>
</tr>
<tr>
<td>Pos. Cut-off</td>
<td>0.312</td>
<td>Pos. Cut-off</td>
</tr>
</tbody>
</table>

CLINICAL TESTING

Patient samples were tested using the IgM ELISA kit at three sites: Center for Disease Control and Prevention in China, a University Hospital in China, and a laboratory in the United States. The combined cohort consisted of normal healthy patients with samples collected prior to the COVID-19 outbreak (N=153) and RT-PCR confirmed positive patients (N = 42). The results are as follows:

<table>
<thead>
<tr>
<th></th>
<th>Confirmed Positive</th>
<th>Confirmed Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDI™ Novel Coronavirus</td>
<td>Positive</td>
<td>30</td>
</tr>
<tr>
<td>COVID-19 IgM ELISA Kit</td>
<td>Negative</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>41</td>
</tr>
<tr>
<td>PPA</td>
<td>73.8%</td>
<td>95% CI (Wilson’s Score): 0.581-0.843</td>
</tr>
<tr>
<td>NPA</td>
<td>100%</td>
<td>95% CI (Wilson’s Score): 0.976-1.000</td>
</tr>
</tbody>
</table>

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 some of the clinical testing 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.

REFERENCES


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.

This product is manufactured by

Epitope Diagnostics, Inc.
7110 Carroll Road
San Diego, CA 92121, US

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

Authorized Representative in Europe

MDSS GmbH
Schiffgraben 41
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