**EDI™ COVID-19 Nucleocapsid IgG Quantitative ELISA Kit**

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

### INTENDED USE

This kit is for research use only. Not for use in diagnostic procedures. The kit is for the quantitative detection of COVID-19 IgG antibody in human serum. Individuals 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.

### 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. IgG is the most abundantly found immunoglobulin to be produced in response to an antigen and will be maintained in the body after initial exposure for long term response.

### ASSAY PRINCIPLE

This ELISA kit is designed, developed, and produced for the qualitative measurement of the human anti-COVID-19 IgG antibody in serum. This assay utilizes the microplate based enzyme immunoassay technique.

Assay calibrators, controls, and 1:100 diluted human serum samples are added to the microtiter wells of a microplate that was coated with COVID-19 recombinant full length nucleocapsid protein. After the first incubation period, the unbound protein matrix is removed with a subsequent washing step. A horseradish peroxidase (HRP) labeled polyclonal goat anti-human IgG tracer antibody is added to each well. After an incubation period, an immunocomplex of “COVID-19 recombinant antigen – human anti-COVID-19 IgG antibody - HRP labeled anti-human IgG tracer antibody” is formed if there is specific coronavirus IgG antibody present in the tested specimen. The unbound tracer antibody is removed by the subsequent washing step. HRP-labeled tracer antibody 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 antibody bound to the anti-COVID-19 IgG on the wall of the microtiter well is proportional to the amount of the anti-COVID-19 IgG antibody level in the tested specimen.

### 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 antigen coated Microplate (31217)**

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

2. **COVID-19 IgG Sample Diluent (31218)**

   - **Qty:** 1 x 120 mL
   - **Storage:** 2 – 8°C
   - **Preparation:** Ready to use.

3. **HRP labeled Anti-hIgG Tracer Antibody (31220)**

   - **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 IgG Calibrators Levels 1 - 5 (31250 - 31254)**

   Calibrators with a bovine serum albumin based matrix with non-azide preservative. Refer to vials for exact concentration.
   - **Qty:** 5 x 0.5 mL
   - **Storage:** 2 – 8°C
   - **Preparation:** Ready to use.

8. **COVID-19 IgG Controls (31255 – 31256)**

   Positive and negative controls with a bovine serum albumin based matrix with non-azide preservative.
   - **Qty:** 2 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 from 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 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.

SAMPLE COLLECTION & STORAGE
Only 10 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 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. Sample Preparation
   1. Dilute serum sample by a 1:100 dilution ratio with the COVID-19 IgG Sample Diluent (31218). For each 10 µL of sample, 1000 µL of COVID-19 IgG Sample Diluent (31218) is needed.
   2. Mix well prior to performing the assay.
3. Assay Procedure
   1. Place a sufficient number of microwell strips (31217) in a holder to run the calibrators (31250 - 31254), controls (31255, 31256), 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>Calibrator Level 1</td>
<td>Calibrator Level 5</td>
<td>SAMPLE 2</td>
</tr>
<tr>
<td>B</td>
<td>Calibrator Level 1</td>
<td>Calibrator Level 5</td>
<td>SAMPLE 2</td>
</tr>
<tr>
<td>C</td>
<td>Calibrator Level 2</td>
<td>Control 1</td>
<td>SAMPLE 3</td>
</tr>
<tr>
<td>D</td>
<td>Calibrator Level 2</td>
<td>Control 1</td>
<td>SAMPLE 3</td>
</tr>
<tr>
<td>E</td>
<td>Calibrator Level 3</td>
<td>Control 2</td>
<td>SAMPLE 4</td>
</tr>
<tr>
<td>F</td>
<td>Calibrator Level 3</td>
<td>Control 2</td>
<td>SAMPLE 4</td>
</tr>
<tr>
<td>G</td>
<td>Calibrator Level 4</td>
<td>SAMPLE 1</td>
<td>SAMPLE 5</td>
</tr>
<tr>
<td>H</td>
<td>Calibrator Level 4</td>
<td>SAMPLE 1</td>
<td>SAMPLE 5</td>
</tr>
</tbody>
</table>

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 should include adequate controls with known COVID-19 IgG levels. 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 absorbance for each pair of duplicate test results.
2. The calibration curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.
3. It is recommended to use following curve fits: (1) Point-to-Point, or (2) 4-Parameter or (3) CubicSpline.
4. The COVID-19 IgG concentrations for the controls and patient samples are read directly from the calibration curve using their respective corrected absorbance.
LIMITATIONS OF THE PROCEDURE
1. This kit is for research use only. Not for use in diagnostic procedures.
2. Since there is no Gold Standard calibration available for COVID-19 IgG measurement, the values of the assay calibrators were established by diluting a highly purified human COVID-19 IgG in a protein matrix.
3. In the first week of the onset of the infection with the novel coronavirus (COVID-19), results may be negative for IgG. In additional, low immunity or diseases that affect immune function, important systemic organ failure, or use of drugs that suppress immune functions are conditions that may contribute to negative results of new coronavirus IgG. Previous infection of SARS or other coronavirus strains may cause light IgG positive results due to similarity to other strains.
4. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
5. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

EXPECTED VALUES
One hundred and thirty-one donor serum samples from December 2019 were collected and tested. The range of COVID-19 IgG was from 0.2 U/mL to 25.3 U/mL. The average concentration is 3.9 U/mL with a median at 3.1 U/mL and a standard deviation at 3.1 U/mL. The manufacturer recommended P97.5 positive cut-off level is 10 U/mL. If maximum clinical specificity is desired, the P99 positive cut-off level at 20 U/mL can be used. It is highly recommended that each laboratory should establish their own normal range for COVID-19 IgG based on local populations.

EXAMPLE DATA
This ELISA calculates the concentration values of the samples and the controls by a calibration curve (fitting method: four parameters or point-to-point) and the measured absorbance. The following is a typical calibration curve,

Note: This curve should not be used in lieu of calibrator curve run with each assay.

<table>
<thead>
<tr>
<th>Microwell ID</th>
<th>Reading Absorbance (450 nm)</th>
<th>Concentration (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD Readings</td>
<td>Average OD</td>
</tr>
<tr>
<td>Calibrator Level 1: 0 U/mL</td>
<td>0.474</td>
<td>0.469</td>
</tr>
<tr>
<td>Calibrator Level 2: 6.9 U/mL</td>
<td>1.030</td>
<td>1.029</td>
</tr>
<tr>
<td>Calibrator Level 3: 26.3 U/mL</td>
<td>1.485</td>
<td>1.447</td>
</tr>
<tr>
<td>Calibrator Level 4: 100 U/mL</td>
<td>2.008</td>
<td>1.992</td>
</tr>
<tr>
<td>Calibrator Level 5: 200 U/mL</td>
<td>0.720</td>
<td>0.737</td>
</tr>
<tr>
<td>Control 1</td>
<td>0.754</td>
<td>1.288</td>
</tr>
</tbody>
</table>

PERFORMANCE CHARACTERISTICS
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. It is critical to note that this research is exceptionally limited at present.

Limit of Detection
The limit of detection (LoD) was determined by 14 replicates of both zero standard and level 2 standard, which is to be 0.17 U/mL.

Linearity
Linearity was determined by the duplicate determination of a clinical positive serum sample with a serial dilution using assay buffer.

<table>
<thead>
<tr>
<th>Well ID</th>
<th>Average Concentration (U/mL)</th>
<th>Theoretical Concentration (U/mL)</th>
<th>Linear Recovery (%)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>66.96</td>
<td>66.96</td>
<td>-</td>
<td>0.999</td>
</tr>
<tr>
<td>1:2</td>
<td>32.30</td>
<td>33.48</td>
<td>96.5%</td>
<td></td>
</tr>
<tr>
<td>1:4</td>
<td>16.03</td>
<td>16.74</td>
<td>95.6%</td>
<td></td>
</tr>
<tr>
<td>1:8</td>
<td>7.19</td>
<td>8.37</td>
<td>86.0%</td>
<td></td>
</tr>
<tr>
<td>1:16</td>
<td>3.34</td>
<td>4.18</td>
<td>79.8%</td>
<td></td>
</tr>
</tbody>
</table>

Intra-assay Precision
The intra-assay precision was determined by the measurement of three serum samples in eight replicates. The results are summarized below with satisfactory precision.

<table>
<thead>
<tr>
<th>Well ID</th>
<th>Average Concentration (U/mL)</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>10.38</td>
<td>0.52</td>
<td>5.0%</td>
</tr>
<tr>
<td>Sample 2</td>
<td>21.93</td>
<td>0.66</td>
<td>3.0%</td>
</tr>
<tr>
<td>Sample 3</td>
<td>66.96</td>
<td>4.53</td>
<td>6.8%</td>
</tr>
</tbody>
</table>

Inter-assay Precision
The inter-assay precision was determined by the measurement of the assay controls in three replicates over twelve runs of the assay. The results are summarized below with satisfactory precision.

<table>
<thead>
<tr>
<th>Well ID</th>
<th>Average Concentration (U/mL)</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>17.9</td>
<td>1.223</td>
<td>6.8%</td>
</tr>
<tr>
<td>Control 2</td>
<td>71.1</td>
<td>6.535</td>
<td>9.2%</td>
</tr>
</tbody>
</table>

Interference
Interference was determined by spiking two different concentrations of interferers into negative and positive samples and testing in duplicate. The interpretation of results did not change after spiking materials into the sample.
The viruses tested are as follows:

Confirmed Disease state samples were tested in the kit in duplicate.

The cohort used to estimate the negative percent agreement (NPA) were pre-COVID-19 specimen collected prior to November 2019 (N = 300) or specimen with a confirmed negative disease state by PCR (N = 16) or serological test (N = 20).

The cohort used to estimate the positive percent agreement (PPA), pre-COVID-19 specimen were selected prior to November 2019 (N = 300) or specimen with a confirmed positive disease state by a polymerase chain reaction (PCR) (N = 108) or serological test (N = 36).

Each specimen was tested in duplicate. The PPA, NPA, and the 95% confidence interval (CI) were calculated. This EDI™ COVID-19 Nucleocapsid IgG Quantitative ELISA Kit successfully differentiates positive patients from normal population. The results are as follows:

This kit has not been tested against MERS-CoV or SARS-CoV strains.

Class Specificity

This experiment was intended to differentiate between the IgG and IgM immunoglobulins. Five PCR confirmed COVID-19 patient serum samples were tested in duplicate in qualitative COVID-19 ELISA kits manufactured by Epitope Diagnostics, Inc. (KT-1032 EDI™ Novel Coronavirus COVID-19 IgG, KT-1033 EDI™ Novel Coronavirus COVID-19 IgM). Samples were found to be originally positive for both IgG and IgM. Further testing conducted on these natural samples presented positive results in the EDI™ COVID-19 Nucleocapsid IgG Quantitative ELISA Kit, Protein A/ProSep A gel was then used to remove the total IgG and the treated samples were tested. The results confirm the treated samples to be positive for IgM, but negative in the EDI™ COVID-19 Nucleocapsid IgG Quantitative ELISA Kit. The results demonstrate that this EDI™ COVID-19 Nucleocapsid IgG Quantitative ELISA Kit exclusively detects the IgG subtype and establishes antibody class specificity for IgG. The results are as follows:

Clinical Testing

A study was performed to determine the clinical performance of the EDI™ COVID-19 Nucleocapsid IgG Quantitative ELISA Kit using serum samples (N = 480) from donors in the United States.

The cohort used to estimate the positive percent agreement (PPA) were specimen with a confirmed positive disease state by a polymerase chain reaction (PCR) (N = 108) or serological test (N = 36).

The cohort used to estimate the negative percent agreement (NPA) were pre-COVID-19 specimen collected prior to November 2019 (N = 300) or specimen with a confirmed negative disease state by PCR (N = 16) or serological test (N = 36).

Each specimen was tested in duplicate. The PPA, NPA, and the 95% confidence interval (CI) were calculated. This EDI™ COVID-19 Nucleocapsid IgG Quantitative ELISA Kit successfully differentiates positive patients from normal population. The results are as follows:

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

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.  
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San Diego, CA 92121, US

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

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

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