INTENDED USE
The EDI™ quantitative SARS-CoV-2 neutralizing antibody ELISA Kit is an Enzyme-Linked Immunosorbent Assay (ELISA) kit intended for the quantitative measurement of the neutralizing antibody to SARS-CoV-2 receptor binding domain (RBD) of its spike protein in human serum. The testing is limited to laboratories certified under the CLIA of 1988, 42 U.S.C. 263a, to perform moderate or high complexity tests. IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, in the convalescent stage, and also after COVID-19 vaccination.

For Research Use Only
Not for use in Diagnostic Procedures

SUMMARY OF PHYSIOLOGY
2019 novel coronavirus 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 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 immunoglobin 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 quantitative measurement of the neutralizing antibodies to SARS-CoV-2 receptor binding domain (RBD) of its spike protein in human serum. This assay utilizes the microplate-based enzyme immunoassay technique.

Assay calibrators, controls, and human serum samples are added to the microtiter wells of a microplate coated with streptavidin. Simultaneously, horseradish peroxidase (HRP) labeled COVID-19 recombinant spike protein and biotinylated angiotensin converting enzyme-2 (ACE-2) are added to each well. After the first incubation period, the unbound protein matrix is removed with a subsequent washing step; a complex of "Streptavidin---Biotin-ACE-2---HRP-COVID-19 recombinant spike protein" is formed. If there is specific COVID-19 neutralizing antibody present in the tested specimen, the formation of the above complex is blocked. A color reaction with a substrate solution in a timed reaction is measured in a spectrophotometric microplate reader. The HRP enzymatic activity of the complex on the wall of the microtiter well is inversely proportional to the amount of the COVID-19 neutralizing antibody level in the tested specimen.

REAGENTS: PREPARATION AND STORAGE
The test kit must be stored at 2 – 8°C. All components are stable until expiration date; please see a label on a kit box.

1. Streptavidin Coated Microplate (10040)
   Microplate coated with streptavidin
   Qty: 1 x 96 well microplate
   Storage: 2 – 8°C
   Preparation: Ready to use

2. Biotinylated ACE-2 (31278)
   Biotinylated recombinant ACE-2 protein
   Qty: 1 x 5 mL
   Storage: 2 – 8°C
   Preparation: Ready to use

3. HRP labeled Spike Protein(31279)
   HRP labeled spike protein in a stabilized protein matrix
   Qty: 1 x 5 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 Concentrated. 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. nCoV Neutralizing Antibody Calibrator Level 1 (31281)
   A ready-to-use sample dilution buffer
   Qty: 1 x 15 mL
   Storage: 2 – 8°C
   Preparation: Ready to use

8. nCoV Neutralizing Antibody Calibrator Level 5 (31285)
   Calibrators with a bovine serum albumin based matrix with non-azide preservative
   Qty: 1 x 0.5 mL
   Storage: 2 – 8°C
   Preparation: Lyophilized powder (see Assay Procedure section)
**EDI™ Quantitative SARS-CoV-2 Neutralizing Antibody ELISA Kit**

Enzyme Linked Immunosorbent Assay (ELISA) for the quantitative measurement of the SARS-CoV-2 neutralizing antibody concentration in a human serum.

**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. Keep out of reach skin, eyes and/or clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Exercise Good Laboratory Practices.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Precision single channel pipettes capable of delivering 10 µL, 20 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 mm glass or plastic 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. Calibrated timer.

**SAMPLE COLLECTION & STORAGE**

Only 50 µL of human serum is required for measurement in duplicate. Samples should only be used on the same day or stored below -20°C. 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.
   3. Reconstitute nCoV Neutralizing Antibody Calibrator Level 5 (31285) by adding 0.5 mL deionized water.

2. **Assay Procedure**
   1. Place a sufficient number of microwell strips (10040) in a holder to run the calibrators, controls, and samples in duplicate.

   **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>

3. Add 25µL of calibrators, controls, and unknown samples into the designated microwells.
4. Add 50 µL of HRP labeled spike protein (31279) into each microwell.
5. Add 50 µL of biotinylated ACE-2 (31278) into each microwell.
6. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at room temperature (20-25°C) for 45 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 substrate (10020) into the microwells.
9. Mix gently and cover the plate with aluminum foil. Incubate at room temperature (20-25°C) for 20 minutes.
10. Remove the aluminum foil and add 100 µL of stop solution (10030) into each of the microwells. Mix gently by tapping the plate.
11. Read the absorbance at 450 nm within 10 minutes with a microplate reader.
**EDI™ Quantitative SARS-CoV-2 Neutralizing Antibody ELISA Kit**

Enzyme Linked Immunosorbent Assay (ELISA) for the quantitative measurement of the SARS-CoV-2 neutralizing antibody concentration in a human serum.

**PROCEDURAL NOTES**
1. Calibrator L4 can be made by mixing 200 µL of calibrator L5 with 400 µL of calibrator 1. Calibrator L3 can be made by mixing 200 µL of calibrator L4 with 400 µL of calibrator 1. Calibrator L2 can be made by mixing 200 µL of calibrator L3 with 400 µL of calibrator 1.
2. 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.
3. Keep light-sensitive reagents away from direct light in the original container and should be stored in a dark area avoiding unnecessary exposure to the light.
4. Store any unused antibody-coated strips in the foil ziploc bag with desiccant to protect from moisture.
5. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
6. Incubation time(s) and/or temperature(s) other than those specified in the package insert may affect result(s).
7. Avoid air bubbles in the microwell as it could result in lower binding efficiency and higher CV% of duplicate reading.
8. All reagents should be mixed thoroughly and gently prior to use. Avoid foaming.

**QUALITY CONTROL**
To assure the validity of the results each assay should include adequate controls with known SARS-CoV-2 neutralizing antibody levels. EDI recommends to include own laboratory controls in addition to those provided with the kit.

**INTERPRETATION OF RESULTS**
1. Calculate the average absorbance for each pair of duplicate test results.
2. The calibration curve is generated by the absorbance of all calibrator levels on the ordinate against the calibrator concentration on appropriate computer assisted data reduction program for the calculation of results.
3. It is recommended to use following curve fits: (1) 4-Parameteror (2) Point-to-Point.
4. The SARS-CoV-2 neutralizing antibody concentrations for the controls and patient samples are read directly from the calibration curve using their respective absorbance values.

**LIMITATIONS OF THE PROCEDURE**
1. The values of the assay calibrators were established by diluting human SARS-CoV-2 neutralizing antibody stock in a phosphate buffer protein matrix.
2. Patients with low immunity or other diseases that affect immune function, failure of critical systemic organs, and use of drugs that suppress immune function can also lead to negative results. Previous infection of SARS or other coronavirus strains may present a low-level SARS-CoV-2 neutralizing antibody due to similarity of different strains.
3. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

**EXPECTED VALUES**
One hundred serum samples from December 2018 to February 2019 were collected and tested. The average concentration of SARS-CoV-2 neutralizing antibody was found to be 1.13 U/mL with a median at 0.64 U/mL. The manufacturer recommended P<sub>97.5</sub> cut-off level is 5 U/mL for the presence of neutralizing antibody in test sample. It is highly recommended that each laboratory should establish their own cut-off level based on local population.

**EXAMPLE DATA (Calibration Curve)**
This ELISA calculates the concentration values for neutralizing antibody of samples by a calibration curve (fitting method: four parameters or point-to-point) and the measured absorbance.

The following is a typical calibration curve:

<table>
<thead>
<tr>
<th>Well ID</th>
<th>OD at 450 nm</th>
<th>Average</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator Level 1:</td>
<td>2.296</td>
<td>2.464</td>
<td></td>
</tr>
<tr>
<td>0 U/mL</td>
<td>2.632</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrator Level 2:</td>
<td>1.454</td>
<td>1.404</td>
<td></td>
</tr>
<tr>
<td>3.7 U/mL</td>
<td>1.354</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrator Level 3:</td>
<td>0.595</td>
<td>0.602</td>
<td></td>
</tr>
<tr>
<td>11.1 U/mL</td>
<td>0.608</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrator Level 4:</td>
<td>0.225</td>
<td>0.225</td>
<td></td>
</tr>
<tr>
<td>33.5 U/mL</td>
<td>0.225</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrator Level 5:</td>
<td>0.136</td>
<td>0.133</td>
<td></td>
</tr>
<tr>
<td>100 U/mL</td>
<td>0.130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>1.086</td>
<td>1.088</td>
<td></td>
</tr>
<tr>
<td>5.618</td>
<td>5.618</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 2</td>
<td>0.469</td>
<td>0.463</td>
<td></td>
</tr>
<tr>
<td>17.977</td>
<td>17.977</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: This curve shouldn’t be used in lieu of calibrator curve run with each assay.
**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 the fourteen replications of two levels calibrators (L1 and L2) over the run of two assays and was found to be 0.314 U/mL.

**Linearity**
Linearity was determined by the duplicate determination of a clinical positive serum sample with a serial dilution using assay buffer. The results are summarized below with satisfactory linearity.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Average Concentration</th>
<th>Linear Recovery</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>8.388 U/mL</td>
<td>100.00%</td>
<td>0.9998</td>
</tr>
<tr>
<td>80%</td>
<td>6.721 U/mL</td>
<td>100.16%</td>
<td></td>
</tr>
<tr>
<td>60%</td>
<td>5.037 U/mL</td>
<td>100.08%</td>
<td></td>
</tr>
<tr>
<td>40%</td>
<td>3.334 U/mL</td>
<td>99.37%</td>
<td></td>
</tr>
<tr>
<td>20%</td>
<td>1.546 U/mL</td>
<td>92.16%</td>
<td></td>
</tr>
</tbody>
</table>

**Intra-assay accuracy**
The intra-assay accuracy was determined by measurement of the three serum samples at three different concentrations in fourteen replicates over run of forty-two tests in three assays. The sample was diluted at 1:5, 1:10, and 1:20. The results are summarized below with satisfactory accuracy.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Average Concentration (U/mL)</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27.155</td>
<td>1.252</td>
<td>4.6</td>
</tr>
<tr>
<td>2</td>
<td>12.826</td>
<td>1.014</td>
<td>7.9</td>
</tr>
<tr>
<td>3</td>
<td>6.702</td>
<td>0.609</td>
<td>9.1</td>
</tr>
</tbody>
</table>

**Interference testing**
The kit was evaluated for the identification of result variability because of presence of potential interferences/endogenous substances. The results are as follows:

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>No interference observed</td>
</tr>
<tr>
<td>Lipid</td>
<td></td>
</tr>
<tr>
<td>Bilirubin</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td></td>
</tr>
</tbody>
</table>

**Clinical data**
A study was performed to determine the clinical performance of the EDI™ quantitative SARS-CoV-2 neutralizing antibody ELISA kit using serum samples (N=87) from donors in the United States. This cohort was used to estimate the positive percent agreement (PPA) were specimen with a confirmed positive disease state by PCR. Another cohort was used to estimate the negative percent agreement (NPA) were pre-COVID-19 specimen collected prior to November 2019 (N=100). The recommended cut-off value is 5 U/mL. The PPA, NPA, and 95% confidence levels were calculated. The results are as follows:

<table>
<thead>
<tr>
<th>EDI™ quantitative SARS-CoV-2 neutralizing antibody ELISA Kit</th>
<th>Positive Samples</th>
<th>Normal Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>85</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>95</td>
<td>97</td>
</tr>
<tr>
<td>Total</td>
<td>180</td>
<td>100</td>
</tr>
<tr>
<td>PPA: 97.70%</td>
<td>95% CL (Wilson’s Score): 0.9200 – 0.9937</td>
<td></td>
</tr>
<tr>
<td>NPA: 97.00%</td>
<td>95% CL (Wilson’s Score): 0.9155 – 0.9897</td>
<td></td>
</tr>
</tbody>
</table>

**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**
**EDI™ Quantitative SARS-CoV-2 Neutralizing Antibody ELISA Kit**

*Enzyme Linked Immunosorbent Assay (ELISA) for the quantitative measurement of the SARS-CoV-2 neutralizing antibody concentration in a human serum.*

**REF KTR-1036 RUO**

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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

This product is manufactured by

**Epitope Diagnostics, Inc.**

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San Diego, CA 92121, USA

Please visit our website at [www.epitopediagnostics.com](http://www.epitopediagnostics.com) to learn more about our products and services.

**GLOSSARY OF SYMBOLS (EN 980/ISO 15223)**

- **Manufacturer**
- **For Research Use Only**
- **Lot Number**

- **Catalog Number**
- **Read Instructions Before Use**
- **Number of Tests**

- **Store at**
- **Use by**
- **Keep Away from Heat and Direct Sunlight**