EPITOPE DIAGNOSTICS, INC.

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

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[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 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]. 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[5].

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 reservative
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)

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9. nCoV Neutralizing Antibody Controls (31286 – 31287)

Controls with a bovine serum albumin-based matrix with non-azide preservative

Qty: 2 x 0.5 mL
Storage: 2 – 8°C.
Preparation: Lyophilized powder (see Assay Procedure section)

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.

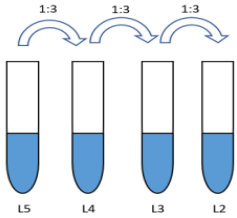
SAMPLE COLLECTION & STORAGE

Only 50 µL of human serum is required for measurement in duplicate. Samples should only be used on the same day or stored below -20ºC. 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.
4. Prepare calibrator level 2, 3 and 4 by 1:3 serial dilutions of level 5 (31285) with nCoV Neutralizing Antibody Calibrator Level 1 (31281). Assay calibrators should be used within 2 hours and should be stored below -20°C. Do not exceed 3 freeze-thaw cycles. The calibrator concentrations are indicated in the certificate of analysis of the kit.



2. Assay Procedure

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

Row	Strip 1	Strip 2	Strip 3
A	Calibrator level 1	Calibrator level 5	Sample 2
B	Calibrator level 1	Calibrator level 5	Sample 2
C	Calibrator level 2	Control 1	Sample 3
D	Calibrator level 2	Control 1	Sample 3
E	Calibrator level 3	Control 2	Sample 4
F	Calibrator level 3	Control 2	Sample 4
G	Calibrator level 4	Sample 1	Sample 5
H	Calibrator level 4	Sample 1	Sample 5

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 (20-25ºC)** 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 (20-25ºC)** 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.

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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.

INTERPRETION 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

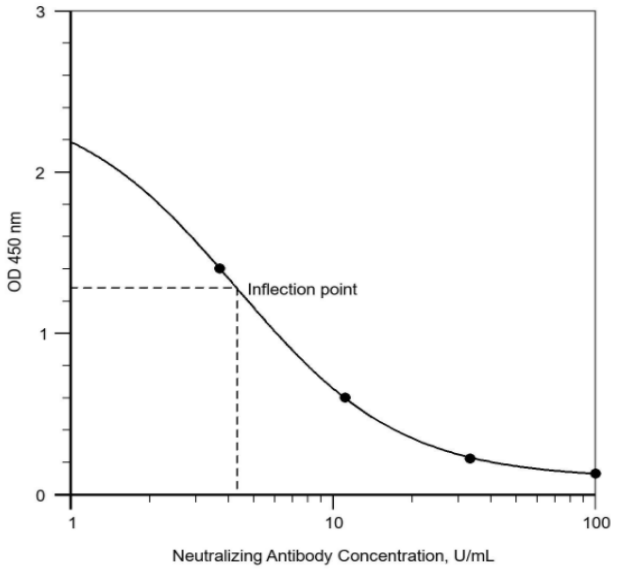
One hundred serum samples from December 2018 to February 2019 were collected and tested. The average concentration of SARS-CoV-2 neutralizing antibody was found to be 1.13 U/mL with a median at 0.64 U/mL. The manufacturer recommended $P_{97.5}$ cut-off level is **5 U/mL** for the presence of neutralizing 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:

Well ID	OD at 450 nm	Average	Concentration
Calibrator Level 1: 0 U/mL	2.296	2.464	
	2.632		
Calibrator Level 2: 3.7 U/mL	1.454	1.404	
	1.354		
Calibrator Level 3: 11.1 U/mL	0.595	0.602	
	0.608		
Calibrator Level 4: 33.5 U/mL	0.225	0.225	
	0.225		
Calibrator Level 5: 100 U/mL	0.136	0.133	
	0.130		
Control 1	1.085	1.088	5.618
	1.090		
Control 2	0.469	0.463	17.977
	0.457		



Note: This curve shouldn't be used in lieu of calibrator curve run with each assay.

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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 replicates 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.

Dilution	Average Concentration	Average Concentration	Linear Recovery	R^2
100%	8.388 U/mL	8.388 U/mL	100.00%	0.9998
80%	6.721 U/mL	6.710 U/mL	100.16%	
60%	5.037 U/mL	5.033 U/mL	100.08%	
40%	3.334 U/mL	3.355 U/mL	99.37%	
20%	1.546 U/mL	1.678 U/mL	92.16%	

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.

Sample ID	Average Concentration (U/mL)	SD	CV (%)
1	27.155	1.252	4.6
2	12.826	1.014	7.9
3	6.702	0.609	9.1

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:

Interferent	Result
Hemoglobin	No interference was observed
Lipid	
Bilirubin	
Protein	

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:

EDI™ quantitative SARS-CoV-2 neutralizing antibody ELISA Kit		
	Positive Samples	Normal Samples
Positive	85	3
Negative	2	97
Total	87	100
PPA: 97.70%	95% CL (Wilson's Score): 0.9200 – 0.9937	
NPA: 97.00%	95% CL (Wilson's Score): 0.9155 – 0.9897	

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

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 to learn more about our products and services.

GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

Manufacturer	RUO For Research Use Only	LOT Lot Number
REF Catalog Number	Read Instructions Before Use	Number of Tests
Store at	Use by	Keep Away from Heat and Direct Sun light