EPITOPE DIAGNOSTICS, INC.

EDI™ Quantitative SARS-CoV-2 Spike Protein IgG ELISA Kit

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

REF KTR-1035 RUO

INTENDED USE

The EDI™ quantitative SARS-CoV-2 spike protein IgG ELISA Kit is an Enzyme-Linked Immunosorbent Assay (ELISA) kit for the quantitative measurement of novel COVID-19 human IgG antibody to SARS-CoV-2 in human serum. IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, as well as in the convalescent stage. The assay is validated manually, but can be adapted to an automated instrument.

For Research Use Only
Not for use in Diagnostic Procedures

SUMMARY OF PHYSIOLOGY

2019 novel coronavirus (2019-nCoV or SARS-CoV-2 or 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]. 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 novel COVID-19 human IgG antibody to SARS-CoV-2 in human 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 spike 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 anti-hIgG 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

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

1. nCoV S-Antigen Coated Microplate (31276)
Microplate coated with SARS-CoV-2 recombinant spike protein
Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to use

2. COVID-19 IgG Sample Diluent (31218)
A ready-to-use sample dilution buffer
Qty: 1 x 120 mL
Storage: 2 – 8°C
Preparation: Ready to use

3. HRP Labeled Anti-hIgG Tracer Antibody (31277)
HRP labeled polyclonal goat anti-humanIgG antibody 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 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 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 IgG Calibrators Level 1 – 5 (31250 – 31254)
Calibrators with a bovine serum albumin based matrix with non-azide preservative
Qty: 5 x 0.5 mL
Storage: 2 – 8°C.
Preparation: Ready to use

8. COVID-19 IgG Controls (31255 – 31256)
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 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 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 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. 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 (31276) in a holder to run the calibrators (31250 - 31254), controls (31255, 31256), 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 **20 µL** of calibrators (31250 - 31254), controls (31255, 31256), and 1:100 diluted samples into the designated microwells.
4. Add **100 µL** of sample diluent into each microwell.
5. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25ºC)** 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 Anti-hIgG Tracer Antibody (31277) into the microwells.
8. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25ºC)** 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 HRP substrate (10020) into the microwells.
11. Mix gently and cover the plate with aluminum foil. Incubate at **room temperature (20-25ºC)** for **20 minutes.**
12. Remove the aluminum foil and add **100 µL** of stop solution (10030) into each of the microwells. Mix gently by tapping the plate.
13. Read the absorbance at **450 nm** within **10 minutes** with a microplate reader.

PROCEDURAL NOTES

1. Both calibrators and controls are pre-diluted (no dilution is required) and should be used directly while performing a test.
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 the 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 a duplicate reading.
8. All reagents should be mixed thoroughly and gently prior to use. Avoid foaming.

QUALITY CONTROL

To assure the validity of the test results each assay should include adequate controls with known COVID-19 IgG 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) Point-to-Point, or (2) 4-Parameter.
4. The COVID-19 IgG 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 an inactivated human COVID-19 IgG 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 light IgG concentration due to similarity of different strains.
3. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

EXAMPLE DATA (Standard Calibration Curve)

This ELISA calculates the concentration values for IgG antibodies of samples and controls by a calibration curve (fitting method: four parameters or point-to-point) and measured absorbance values.

The following is a typical calibration curve:

Microwell ID	Reading Absorbance (450 nm)		
	OD Readings	Average OD	Concentration (U/mL)
Calibrator Level 1: 0 U/mL	0.051	0.050	
	0.049		
Calibrator Level 2: 4.1 U/mL	0.102	0.104	
	0.106		
Calibrator Level 3: 16.5 U/mL	0.244	0.241	
	0.238		
Calibrator Level 4: 63.5 U/mL	0.794	0.763	
	0.732		
Calibrator Level 5: 200 U/mL	2.210	2.254	
	2.337		
Control 1	0.170	0.171	10.135
	0.172		
Control 2	0.482	0.476	36.334
	0.464		

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

EXPECTED VALUES

One hundred donor serum samples from 2018 and early 2019 were collected and tested. The range of SARS-CoV-2 Spike Protein IgG was found from 1.587 U/mL to 102.379 U/mL. The average concentration 29.158 U/mL with a median at 25.007 U/mL and a standard deviation at 20.166 U/mL were found. The manufacturer recommended P_{90} positive cut-off level is **60 U/mL.** It is highly recommended that each laboratory should establish their own normal range for COVID-19 IgG based on local populations.

Conversion to WHO Standard

This ELISA kit has studied against the First WHO International Standard for anti-SCARS-Cov-2 (NIBSC code: 20/136). To convert the kit calibrator in the U/mL to WHO standard in BAU/mL, please use the following formula

WHO Sample Value (BAU/mL) = Measured Value x 0.04395

The cut off by using WHO standardized calibration value is 2.0 BAU/mL in this kit.

PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD)

The Limit of Detection (LoD) was determined by 16 replicates of two levels calibrators (L1 and L2) over the run of two assays and was found to be 0.299 U/mL.

Linearity

Linearity was determined by the serial dilutions using assay buffer at 1:02, 1:04, 1:08, and 1:16 of a positive sample serum. Satisfactory test correlation (R^2 = 0.9998) was observed by analyzing measured anti-SARS-CoV-2 IgG concentration against the theoretically calculated IgG concentration using a linear regression.

The results are summarized below with satisfactory linearity.

Well ID	Average Concentration (U/mL)	Theoretical Concentration (U/mL)	Linear Recovery (%)	R^2
Original	120.50	120.50	100%	0.9998
1:02	61.20	60.25	101.58%	
1:04	30.00	30.13	99.59%	
1:08	15.80	15.06	104.90%	
1:16	7.10	7.53	94.27%	

Intra-assay Accuracy

The intra-assay accuracy was determined by the measurement of three serum samples at three different concentrations in fourteen replicates over the run of forty-two tests in three assays.

The results are summarized with satisfactory accuracy.

Sample	Average Concentration(U/mL)	SD	CV (%)
1	60.17 U/mL	3.86	6.41 %
2	64.50 U/mL	2.90	4.50 %
3	97.41 U/mL	4.80	4.93 %

Inter-assay Reproducibility

The inter-assay reproducibility was determined by the measurement of serum samples (light and strong positive) in three replicates over the thirty-six tests in twelve assays.

The results are summarized below with satisfactory reproducibility.

Sample	Average Concentration(U/mL)	SD	CV (%)
1	10.10 U/mL	0.67	6.54 %
2	40.21 U/mL	2.56	6.36 %

Interference

The EDI™ Quantitative SARS-CoV-2 Spike protein IgG kit was evaluated for identification of result variability because of presence of potential interferences/endogenous substances.

The results are as follows:

Interferents	Result
Hemoglobin	No interference was observed
Lipid	
Bilirubin	
Protein	

Class Specificity

This test is intended to differentiate between the IgG and IgM immunoglobulins. Five PCR confirmed COVID-19 patient serum samples were tested in duplicate in EDI™ quantitative SARS-CoV-2 spike protein IgG ELISA kit before and after ProSep-A extraction procedure in a single assay.

The results are as follows:

Sample ID	Before ProSep-A Extraction (U/mL)	After ProSep-A Extraction (U/mL)
190679	169.0 U/mL	4.8 U/mL
190779	219.8 U/mL	2.7 U/mL
190780	89.7 U/mL	2.7 U/mL
190784	258.2 U/mL	78.6 U/mL
190790	289.7 U/mL	19.8 U/mL

Clinical Testing

A study was performed to determine the clinical performance of the EDI™ quantitative SARS-CoV-2 spike protein IgG ELISA Kit using serum samples (N=87, convalescent plasma samples) 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 a polymerase chain

reaction (PCR). Another cohort 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 60 U/mL. The PPA, NPA, and 95% confidence levels (CL) were calculated.
The results are as follows:

EDI™ Quantitative SARS-CoV-2 Spike Protein IgG ELISA Kit		
KTR-1035	Positive Samples	Normal Samples
Positive	82	9
Negative	5	91
Total	87	100
PPA: 94.25%	95% CL (Wilson's Score): 0.8724 – 0.9752	
NPA: 91.00%	95% CL (Wilson's Score): 0.8377 – 0.9519	

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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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


Use by


Number of Tests


Store at


Read Instructions Before Use


Keep Away from Heat and Direct Sun light