Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it

EPITOPE DIAGNOSTICS, INC.

FOR REFERENCE USE ONLY

EDI™ COVID-19 Nucleocapsid IgG Quantitative ELISA Kit

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

REF KT-1034 IVD CE 96

INTENDED USE
The kit is produced for the quantitative measurement of novel COVID-19 IgG antibody concentration in human serum.

This kit is for in vitro diagnostics use only.

INTENDED USER
For laboratory professional use or healthcare professionals only

SUMMARY OF PHYSIOLOGY
2019 novel coronavirus (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 qualitative measurement of 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)
Microplate coated with COVID-19 recombinant 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 (31220)
HRP labeled polyclonal goat anti-hIgG 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 preservative.
Qty: 1 x 30 mL
Storage: 2 – 25°C
Preparation: 30X Concentrated. Must be diluted with 870 mL distilled water and mixed well before use.

5. ELISA HRP Substrate (10020)
Tetramethylbenzidine (TMB) with a 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 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)
Controls with a bovine serum albumin based matrix with non-azide preservative. Refer to vials for exact concentration.
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, 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 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. Severely hemolyzed 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 (31217) 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 (20-25 ºC)** 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 (31220) into the microwells.
8. Mix gently and cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** 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 substrate (10020) into the microwells.
11. Mix gently and cover the plate with aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
12. Remove the aluminum foil and add **100 µL** of stop solution (10030) into each of the microwells. Mix by gently by tapping the plate.
13. 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 away from direct light in the original container and should be stored in a dark area avoiding unnecessary exposure to the light.
3. Store any unused antibody-coated strips in the foil ziploc bag with desiccant to protect from the moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation time(s) and/or temperature(s) other than those specified in the package insert may affect result(s).
6. Avoid air bubbles in the microwell as it could result in lower binding efficiency and higher CV% of a duplicate reading.
7. All reagents should be mixed thoroughly and gently prior to use. Avoid foaming.

QUALITY CONTROL

It is recommended that all assays include the laboratory's own controls in addition to those provided with this 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. Since there is no Gold Standard concentration 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.
2. In the first week of the onset of the infection with the novel coronavirus (COVID-19) patients results may be negative for IgG. 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 IgG. Previous infection of SARS or other coronavirus strain may cause a light IgG positive in view of similarity of different strains.
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. For samples that do not align with PCR testing, perform a confirmation test by retesting the sample 2-3 times.
6. The assay is validated manually, but can be adapted to an automated instrument.

EXPECTED VALUES

One hundred 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 $P_{97.5}$ positive cut-off level is **10 U/mL**. If maximum clinical specificity is desired, the P_{99} 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 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	Concentration (U/mL)
Calibrator Level 1: 0 U/mL	0.074	0.072	
	0.070		
Calibrator Level 2: 6.9 U/mL	0.474	0.469	
	0.464		
Calibrator Level 3: 26.3 U/mL	1.030	1.029	
	1.028		
Calibrator Level 4: 100 U/mL	1.485	1.447	
	1.410		
Calibrator Level 5: 200 U/mL	2.008	1.992	
	1.976		
Control 1	0.720	0.737	16.188
	0.754		
Control 2	1.323	1.288	71.893
	1.253		

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 14 replicates of both zero standard and level 2 standards, and was found 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.

The results are as follows:

Well ID	Average Concentration (U/mL)	Theoretical Concentration (U/mL)	Linear Recovery (%)	R^2
Original	66.96	66.96	-	0.999
1:02	32.30	33.48	96.5%	
1:04	16.03	16.74	95.6%	
1:08	7.19	8.37	86.0%	
1:16	3.34	4.18	79.8%	

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.

Sample	Average Concentration (U/mL)	SD	CV (%)
1	10.38	0.52	5.0%
2	21.93	0.66	3.0%
3	66.96	4.53	6.8%

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.

Control	Average Concentration (U/mL)	SD	CV (%)
1	17.9	1.223	6.8%
2	71.1	6.535	9.2%

Interference

Interference was determined by spiking two different concentrations of interferents into negative and positive samples and testing in duplicate. The interpretation of results did not change after spiking materials into the sample.

Interferent	Result
Hemoglobin	No interference was found
Lipid	
Bilirubin	
Protein	

Cross-Reactivity

A large number of known negative samples (300 unique samples collected in the US prior to December 2019) were tested from a population with a high prevalence of infection with, and/or vaccinated against the following viruses. A specificity of 98.3% was observed. Cross-reactivity testing for the below listed viruses is not expected.

Anti-influenza A (IgG and IgM)	Anti-229E (alpha coronavirus)
Anti-influenza B (IgG and IgM)	Anti-NL63 (alpha coronavirus)
Anti-HCV (IgG and IgM)	Anti-OC43 (beta coronavirus)
Anti-HBV (IgG and IgM)	Anti-HKU1 (beta coronavirus)
Anti-haemophilus influenzae (IgG and IgM)	Anti-respiratory syncytial virus (IgG and IgM)
Antinuclear Antibody	Anti-HIV

Confirmed disease state samples were tested in the kit in duplicate. The viruses tested are as follows:

Virus	Confirmation Test	Sample ID	Concentration (U/mL)	Results
Respiratory Syncytial Virus (IgG)	EIA, Viron/Serion	1	5.9	Negative
		2	8.3	Negative
		3	3.6	Negative
		4	7.8	Negative
		5	6.0	Negative
Influenza A (IgG)	Viron/Serion	1	3.8	Negative
Influenza B (IgG)	Viron/Serion	1	4.4	Negative
HIV	-	1	2.8	Negative
Hepatitis B Virus	Siemens	1	4.8	Negative
		2	2.7	Negative
		3	2.3	Negative
		4	6.2	Negative
		5	2.9	Negative

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, and 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:

Sample ID	Replicate	Concentration (Natural, NO Treatment)	Result (Natural, NO Treatment)	Result Agreement	Concentration (With Treatment)	Result (With Treatment)	Expected Result (With Treatment)	Result Agreement
190679	1	170.680	+	✓	9.503	-	-	✓
	2	168.405	+	✓	8.808	-	-	✓
190790	1	201.203	+	✓	2.114	-	-	✓
	2	198.568	+	✓	1.687	-	-	✓
190779	1	196.055	+	✓	8.864	-	-	✓
	2	189.925	+	✓	7.079	-	-	✓
190784	1	161.039	+	✓	6.342	-	-	✓
	2	163.284	+	✓	5.527	-	-	✓
190780	1	62.689	+	✓	1.103	-	-	✓
	2	64.646	+	✓	0.666	-	-	✓

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 = 20). Each specimen was tested in a 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:

KT-1034		Confirmed Positive	Confirmed Negative
EDI™ COVID-19 Nucleocapsid IgG Quantitative ELISA Kit	Positive	130	9
	Negative	14	327
	Total	144	336
PPA: 90.3 %	95% CI (Wilson's Score): 0.843 – 0.941		
NPA: 97.3 %	95% CI (Wilson's Score): 0.949 – 0.986		

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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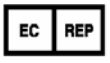
TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

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.
7110 Carroll Road
San Diego, CA 92121, USA

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



MDSS GmbH
Schiffgraben 41,
30175 Hannover, Germany

GLOSSARY OF SYMBOLS (EN 980/ISO 15223)